



# A MANUAL OF BIOCHEMISTRY

BY

J. F. McCLENDON

*Professor of Physiological Chemistry*  
*University of Minnesota Medical School*

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THIS BOOK IS DEDICATED TO

A. P. MATHEWS

WHOSE LECTURES IN WOODS HOLE  
WERE MY FIRST INSPIRATION IN  
BIOCHEMICAL WORK .



## PREFACE

The present work is a condensation and rewriting of mimeographed material with considerable additions. Many of the data have been condensed into a table at the end of this book. This table lists over a thousand chemical substances which are of interest in the related fields of biochemistry. It is found very difficult to separate physiology from pharmacology, pathology, and bacteriology, and no attempt was made to do so in the table, which contains biological as well as chemical data.

The main bulk of the mimeographed text has been condensed to an arrangement which makes easier reading, but it includes far more data on the biochemistry of inorganic substances and certain organic groups than are found in textbooks of physiological chemistry. The attempt was made to arrange substances in their chemical order (with the physiological order in the summary) but it was found very difficult to separate sodium from chlorine, for instance, since they exist chiefly as sodium chloride, and calcium from phosphorus since they exist chiefly in the body as minerals of the apatite series, or to separate enzyme or hormone from "substrate."

Owing to the huge number of substances that occur in our food and therefore may either be absorbed or attacked by enzymes, and the fact that textbooks of biochemistry have never classified many of these substances, a section on fermentative products and essential oils (including related substances of animal origin) embraces quite a number of these substances. This section is intended to illustrate the process of fermentation and the synthetic powers of plants as well as to include many essential physiological substances, such as vitamin D, vitamin A, theelin, and theelol, which usually are not grouped according to their chemical nature.

In the section on biophysical chemistry there are included enzyme action and the effect of hormones on metabolism, and in this section are listed the hormones, particularly those which are of unknown chemical composition. Since several of the vitamins

are of known composition a section on vitamins is not included but vitamins are listed in the introduction and individual ones of known chemical constitution are considered in other places in the book.

I wish to express my indebtedness to Drs. Allan Hemingway, R. H. Hamilton, Jr., J. W. Cavett, and W. D. Armstrong, to Messrs. Harold Lundgren, Harold Street, and Frank Naegeli, and to Miss Pearl Olson for assistance in preparing this book for the press.

J. F. McCLENDON.

MINNEAPOLIS, MINNESOTA,

*January 1, 1934.*

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# A MANUAL OF BIOCHEMISTRY

## PART I

### INTRODUCTION

Physiological chemistry refers generally to that phase of biochemistry that has been developed in medical schools — in fact, it is an outgrowth of the physiology which has developed in medical schools. Pathological chemistry has developed as part of the physiological chemistry. As a branch of physiology, physiological chemistry concerns itself with the chemistry of living cells and cell aggregates known as tissues, as well as non-cellular products, tissue fluids and secretions, such as the spinal fluid and the urine.

Weight of elements in a person weighing 50,000 grams:

Oxygen.....	33,000	Potassium.....	200
Carbon.....	8,750	Sodium.....	150
Hydrogen.....	5,100	Chlorine.....	150
Nitrogen.....	1,200	Sulfur.....	100
Calcium.....	800	Magnesium.....	25
Phosphorus.....	450	Iron.....	3

Traces of Li, I, Br, F, Si, Cu, Mn, As, Ag, Pb.

The most abundant compound in the body is water.

Carbon exists only in compounds, chiefly with oxygen, hydrogen, and often with nitrogen. Compounds of carbon are called organic compounds. About one-third of the body consists of them. When it was thought that all organic compounds were produced by living things, carbon dioxide was not considered an organic compound because it was known to occur in geological formations. Carbon dioxide and water unite with some of the sodium of the blood to form sodium bicarbonate which is of great importance since it has the power of neutralizing strong acids, keeping the blood neutral. Carbon is eliminated from the body chiefly as carbon dioxide and is given off through the lungs, although some of the carbon dioxide is united with ammonia to

form urea which goes out through the urine. If heated, some organic compounds char, leaving black charcoal.

Most of the hydrogen and oxygen in the body are in the form of water. Hydrogen in the ionic form, or hydrogen ions, is given off by all acids. The most acid region in the body is in the stomach (gastric juice). Carbon, hydrogen, and oxygen are united to form carbohydrates most of which are sugars and are absorbed from the food and circulated in the blood.

On oxidation, sugars may give rise to organic acids such as acetic; and when these unite with nitrogen in the form of ammonia, they are called amino acids. Amino acids have the power of combining together to form very large molecules known as proteins. Familiar examples of proteins are materials for clothing (wool, silk, and leather). Sulfur is a constituent of some of the amino acids and proteins. The phosphorus in the body is primarily in the form of phosphoric acid; but this may be united with other substances, for instance proteins.

Some carbon, hydrogen, and oxygen are in the form of fats, which are ordinarily compounds of fatty acids and glycerol. Some compounds of glycerol and fatty acids unite also with phosphoric acid and nitrogenous bases and are called phosphatides. Similar substances contain sugar and are called cerebroside because they occur in the brain.

Chemical changes taking place in organisms are collectively known as metabolism. With the development of thermodynamics in chemistry, the energy-exchange becomes important; and this is called calorimetry. So far as weight of constituents is concerned, the most important in the body is first water, then protein, fat, and carbohydrate. Although, of these three, protein usually forms the greatest percentage, fat is very variable and may increase until it is greater in weight than protein.

Protein is considered important in the study of kidney disease (nephritis). Protein (albumin) occurs in the urine in nephritis and may then become deficient in the blood plasma. Therefore, it is believed by many that albumin passes from the blood to the urine in nephritis. The edema (dropsy, too much water in the tissues) of nephritis as well as other diseases is associated with a low concentration of albumin in the blood plasma.

Carbohydrate is always the lowest of these three constituents in weight. The study of carbohydrate is important in diabetes

mellitus (flow of sweet urine) as in this disease it passes out in the urine, and the body cannot use it for fuel.

These three constituents of the body are also the chief constituents of foods besides water, and the calorific value when burned in the body is usually given as: protein, 4.1 kilogram-calories per gram; carbohydrate, 4.1; and fat, 9.3. The combined energy from the burning of these three substances (when not used for muscular work) is given out as heat and is used in maintaining the body temperature.

**Vitamins.** Biochemistry has made nutrition almost a science; that is to say, the chemical nature of the inorganic elements, fats, and carbohydrates is known and many proteins have been crystallized and their products of hydrolysis are known; but there remains a group called vitamins which is necessary in nutrition and whose chemical nature is still somewhat uncertain. Therefore, they have been named vitamin A, B, C, D, E, G.

Vitamin A is supposed to be related to the terpenes and is listed under that group, being a derivative of

another terpene compound, carotin, which is the yellow color of plants. Lack of vitamin A causes a dryness of the eyes known as xerophthalmia (fig. 39). It also causes keratization of the lining of the female genital tract and sterility. Its presence not only prevents these and other specific morbidities but improves the general health and is said to aid in the resistance to infections.

Vitamins B ( $C_6H_{10}ON_2$ ) and G are mixed in many preparations; wheat germ contains very little G but is a source of B. The absence of vitamin B produces polyneuritis (figs. 1, 2), which has been called beriberi and is known as "kakke" in Japan, where it was

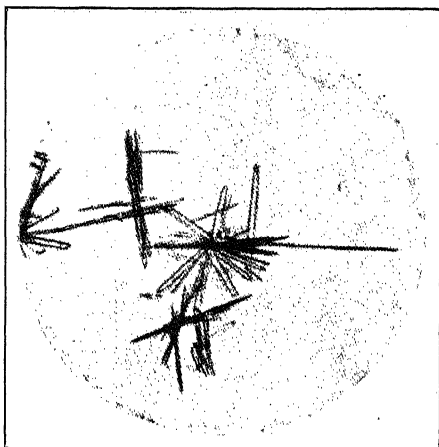


FIG. 1. Vitamin B hydrochloride crystals.  
Jansen and Donath.

first prevented in the navy by dietary means by Baron Takaki. The presence of the vitamin is said not only to prevent or cure beriberi but also to improve many life processes and to promote the appetite. Absence of G is shown in fig. 3.

Jansen and Donath: *Med. Dienst Volksgesondheid Ned.-Indie* 1:1 (1927).

Vitamin C is ascorbic acid, one of the fatty acids, and is listed in that group. Apparently it can be synthesized by the rat, but it is necessary for the guinea-pig and man. It is best sup-

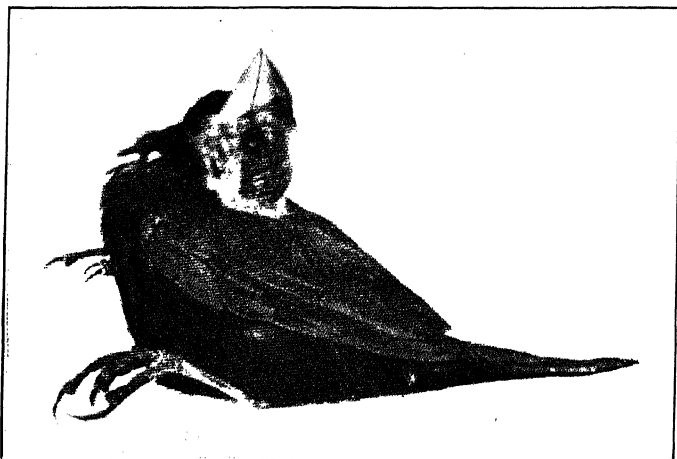


FIG. 2. Avian polyneuritis. Jansen and Donath.

plied in acid fruits, in which it stands cooking and drying. Lack of it causes scurvy (figs. 4, 5). In scurvy, the bones and teeth are decalcified and the products pass out in the urine. The osteoporosis of the bones leads to fractures, which are prominent in children at the costochondral junctions of the ribs, and healing leads to a beading simulating rickets. The cementum is the first part of the teeth to go, leading to the dropping out of the teeth. Lack of vitamin C causes the endothelial cells of the capillaries to separate and leads to hemorrhage. Vitamin C is easily destroyed by oxidation, but the acids of fruits lessen its rate of oxidation. It is absent in spray-dried milk. Copper in condensed milk aids in its oxidation. Spray drying in flue-gas may preserve it (figs. 6, 7).

Hess: *Scurvy Past and Present*, Lippincott, Philadelphia, (1920).

Vitamin D is considered to be an isomere of ergosterol and is listed with the terpenes. It may be absorbed by the alimentary tract whereas ergosterol is not, but ergosterol is synthesized in the body and is changed by ultra-violet light, penetrating the skin, into vitamin D. It is not shown to be necessary, but a double deficiency causes morbidity, a deficiency of vitamin D and phosphate causing rickets (fig. 27) and a deficiency of vitamin D and calcium causing osteoporosis (fig. 26). These are characterized mainly by defects in the bones and teeth. Rickets is accompanied by a low blood phosphate level



FIG. 3. Pellagra. Merck.



FIG. 4. The leg in scurvy. Hess, Scurvy Past and Present.

whereas osteoporosis may be accompanied by a low blood calcium level, in which case there is also tetany or increased excitability of the nerves. Cod-liver oil is a good source of vitamins A and D. Vitamin D may now be purchased in purer form under the name of "viosterol."

Lack of vitamin E causes sterility in the male and absorption of the fetus in the female. It is one of the so-called fat-soluble vitamins, which include A, D, and E, and therefore may be related to the terpenes, but that has not yet been suggested except for the statement that it

occurs in the non-saponifiable fraction of wheat-germ oil.

Evans and Burr: Mem. Univ. Calif. 8:9 (1927).

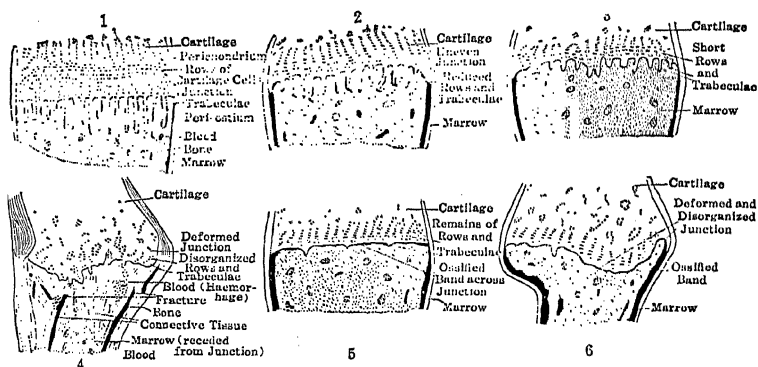


FIG. 5. The costochondral junction. Delf and Tozer; 1, normal; 2, incipient scurvy; 3, definite scurvy; 4, fracture due to scurvy; 5, severe scurvy; 6, healed fracture causing "beading" of ribs.

Vitamin G is included in the water-soluble vitamins, which include B, C, and G, and is often mixed with them in vitamin preparations. It is heat-

stable, whereas C is very rapidly destroyed by oxidation and heat increases oxidation, and B is destroyed by one hour of autoclaving at about 20 pounds of pressure. Yeast is a good source of vitamins B and G; vitamin B may be destroyed by autoclaving, leaving the autoclaved yeast as a good preparation of G. The lack of vitamin G in man produces pellagra or rough skin (fig. 3) but only if the skin is exposed to sunlight — in other words it intensifies sunburn. Pellagra is accompanied by alimentary and nervous disturbances. Vitamin G is probably

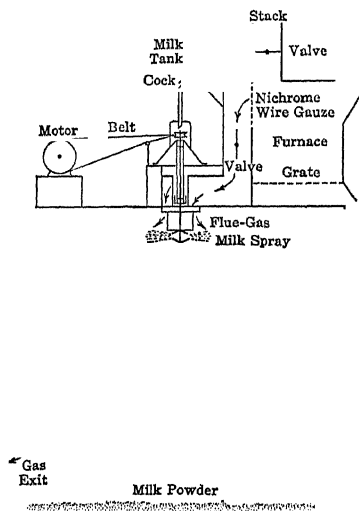
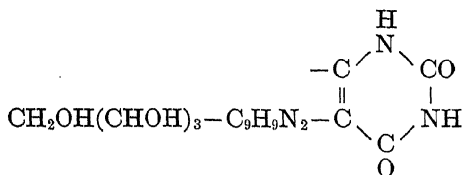


FIG. 6. Drier for preserving vitamins.  
Journal of Biological Chemistry.

mentary and nervous disturbances.



Kuhn, Rudy and Wagner-Jauregg: Ber. 66:1950 (1933).

U. S. Pub. Health Service, Hygienic Lab. — Bull. 120 (1920).

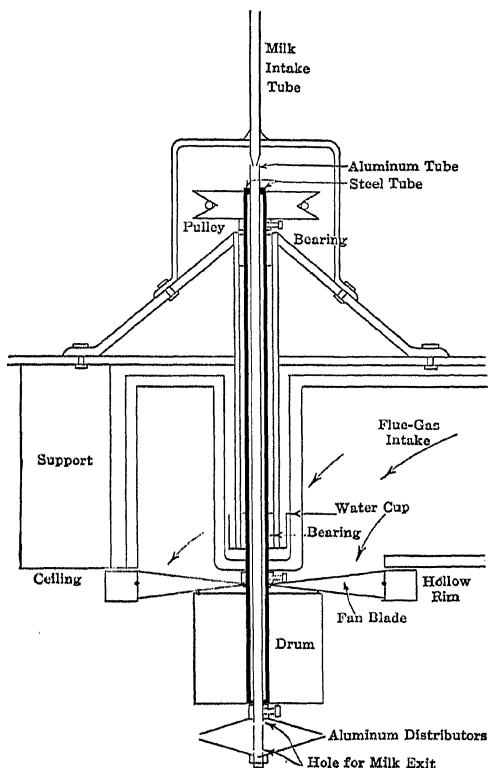


Fig. 7. Detail of the above drier. Journal of Biological Chemistry.

For further data on the vitamins, see the proper sections in case the chemistry is known.

Bodansky: Introduction to Physiological Chemistry, third edition, John Wiley & Sons, Inc., New York (1934).

## DIVISION 1

## MOLECULAR SIZE, COLLOIDS, AND CRYSTALLOIDS

Thomas Graham divided molecules according to size by the method of dialysis, i.e., passage through a porous membrane that acted as a molecule-sieve. He found that glue (gelatin) would not diffuse through such membranes as bladder and named substances which behaved similarly "colloids" from the Greek *kolla* = glue. Solutions of colloids were called "sols."

Von Wiemarn showed that any solid substance may be made into a sol by finely dispersing it in a solvent in which it was very slightly soluble or insoluble. Thus, gold sols could easily be prepared by dispersing gold in water. Special mills for dispersing solids in liquids have been made for the preparation of sols. In such sols, the colloid particles have little affinity for the solvent (dispersing medium) and are called *lyophobic colloids*; if water is the dispersing medium, they are called hydrophobe colloids. Lyophobic colloid particles are found to be electrically charged; and, if the charge is neutralized, they aggregate and precipitate.

Glue, on the other hand, may not always precipitate if the electric charge on its particles is neutralized, as the particles have a great affinity for the dispersing medium; hence glue and similar colloids are called *lyophile* (or, in case water is the dispersing medium, *hydrophile*, water-loving). Sørensen has shown that certain hydrophile sols are in true solution in the sense that a colloid particle consists of a single molecule, but the molecular weight is enormous and the particle size may be as large as one of a hydrophobe colloid, that may consist of hundreds of molecules.

Colloids are distinguished by the fact that they have surfaces in the ordinary physical sense, and colloid chemistry is largely surface chemistry, i.e., chemistry of heterogeneous systems. The colloid (disperse phase) is dispersed in a fluid (the dispersing medium), and if the colloidal particles are separate the system is called a colloidal solution or *sol*, but if they touch one another so as to inhibit the movement of each other the system may be a colloidal jelly or *gel*. The sol-gel transformation may take place slowly without change in concentration or temperature, a process known under the name of *hysteresis*.

The size of the particles in a sol is usually between  $0.1\mu$  and  $5m\mu$ . (One micron [ $\mu$ ] is one-millionth of a meter or one-thousandth of a millimeter, and one millimicron [ $m\mu$ ] is one-thousandth of a micron or one-millionth of a millimeter.) If the refractive indices of particle and medium are sufficiently different, particles as small as  $0.25\mu$  may be seen in the ordinary compound microscope. By powerful indirect illumination (ultramicroscope) smaller particles may be seen as stars against a black sky down to  $5m\mu$  in diameter and are called submicrons. Still smaller particles (amicros) when numerous give a haze (like the Milky Way) in the ultramicroscope. Submicrons out of focus also give such a haze; the presence of amicros is not ascertained in this manner unless larger particles are absent. Sols with very small particles appear red, with larger particles they appear blue, with still larger particles the color of the substances in mass may have an effect.

Particles in suspension exhibit *Brownian movement*, owing to unequal bombardment by molecules of the medium. Large particles show merely an oscillatory motion, but submicrons show zigzag, translatory movements. The movement increases with the temperature and decreases with the viscosity of the medium.

Bancroft: Applied Colloid Chemistry, McGraw-Hill, New York (1921).

Bayliss: The Colloidal State in its Medical and Physiological Aspects, Oxford Univ. Press, New York (1923).

Bechhold: Die Kolloide in Biologie und Medizin, Dresden (1912).

Bogue: Colloidal Behavior, McGraw-Hill, New York (1924).

Burton: The Physical Properties of Colloidal Solutions, Longmans, Green & Co., New York (1916).

Freundlich: The Elements of Colloidal Chemistry, Chemical Age, 12:27 (1925).

Hatschek: The Foundation of Colloid Chemistry, E. Benn, Ltd., London.

Ostwald: Grundriss der Kolloidchemie, Steinkopf, Dresden (1905).

Zsigmondy: Kolloidchemie, Lehrbuch, third edition, Leipzig (1921).

**Lyophobic Colloids.** By means of various dispersion methods, any substance may be finely dispersed (in a fluid which will not dissolve all of it) and made into a colloidal solution. Such sols are usually lyophobic, i.e., the colloid has weak affinity for the dispersing medium and is not solvated to an appreciable extent. Lyophobic sols are usually distinct in the ultra-microscope, appear strongly colored, are of low viscosity, and are easily precipitated by electrolytes.

Fine emulsions of fat behave as hydrophobic colloids.

Agents which tend to disperse colloids are called peptizing agents.

Svedberg: The Formation of Colloids, Van Nostrand, New York (1921).

**Lyophile Colloids.** The higher members of the series of proteins, fats, and carbohydrates have very great molecular weights and possess colloid properties even when molecularly dispersed because the molecules are of the dimensions of colloid particles. Such substances form *sols* and *gels* and diffuse with difficulty into gels or through colloidal membranes, and in this way may be separated from substances of smaller molecules known as crystalloids. The colloids of a protein or carbohydrate nature have a strong affinity for water and are classed as *hydrophile* colloids. When proteins crystallize, there is water between the carbon chains in the crystal lattice; and, when this water is evaporated out, the crystals shrivel. Colloidal carbohydrates, such as gum-arabic, agar, and starch, are of more importance to plants (in which they form supporting and storage structures) than to animals.

Although the form of protein molecules or that of any other organic compound cannot be distinguished under the microscope, a good deal of evidence exists to show that organic compounds of high molecular weight have molecules of considerable length. This is brought out by the study of lubricating oils, which are used to separate the moving surface of one metal from another. If the metal surfaces come in contact, the attempt at lubrication is a failure. If too much weight is applied to the bearing, the lubricant breaks down; and it is then found that the long molecules of the oil are broken into shorter ones. In fact, molecules are torn into pieces between the two metal surfaces. Another example is native rubber. If the dried latex is placed in benzene, it will absorb all the benzene and a jelly-like mass of benzene in rubber will be formed, acting as a huge, benzene-soaked, molecule. If, however, a piece of the dried latex is placed between steel rollers and stretched beyond the elastic limit, the long carbon chains in the rubber are torn; and, if this milled rubber is placed in benzene, it forms a colloidal solution. The more the rubber is milled, the smaller the molecules become.

The magnitude of the molecular weights of hydrophile colloids may be illustrated by the following table of proteins.

PROTEIN	MOLECULAR WEIGHT (Cohn)	PROTEIN	MOLECULAR WEIGHT (Cohn)
Gelatin.....	123,600	Glutenin.....	108,900
Zein.....	97,000	Fibrin.....	42,000
Gliadin.....	125,000	Serum albumin.....	45,000
Edestin.....	116,000	Serum globulin.....	81,000
Egg albumin.....	33,400	Hemoglobin, horse.....	66,800

Lyophile colloids may be invisible in the ultra-microscope by reason of similarity of refractive indices of particle and medium.

Lyophilic colloids easily form gels. Gels may "weep" owing to separation of some of the medium, a process known as *syneresis*; for example a blood-clot contracts and serum exudes from it.

Gels and sols show *hysteresis* or slow change with time. The slow formation of a gel such as gelatin or pectin jelly is an example of hysteresis.

Cohn, Hendry, and Prentiss: Studies in the physical chemistry of the proteins, V. The molecular weights of the proteins, I. The minimal molecular weights of certain proteins, *J. Biol. Chem.* 63:721 (1925).

Katz and Mark: Changes in fiber X-ray diagrams of cellulose due to swelling in concentrated aqueous solutions, *Z. physik. Chem.* 115:385 (1925).

Lecomte du Noüy: The probable dimensions of the molecule and molecular weight of crystalline egg albumin, *J. Biol. Chem.* 64:595 (1925).

Pauli: The colloid chemistry of the proteins, *Kolloid Z.* 31:252 (1922); also as book, Dresden (1920).

Svedberg and Fahraeus: New method for the determination of the molecular weight of the proteins, *J. Am. Chem. Soc.* 48:430 (1926).

— and Nichols: The molecular weight of egg albumin, I. In electrolyte free condition, *J. Am. Chem. Soc.* 48:3081 (1926).

**Adsorption and Surface Tension.** Although proteins may act as ampholytes, this action is very weak owing to the small number of free amino and carboxyl groups. The protein molecule has a large part of its surface not covered by polar groups. Adsorption may occur on this surface as well as on the surface of colloid particles in general.

It was shown mathematically by Willard Gibbs that solutes which lower the surface tension of the solution concentrate at the surface. If this surface is the surface of a colloidal particle, the solute is adsorbed by the particle. According to Bayliss, combination between an enzyme and substrate is an adsorption compound.

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Substances which do not reduce the surface tension may be adsorbed.

Lecompte du Noüy: Surface Equilibria of Colloids, Chemical Catalog Co., New York (1926).

**Electric Charge.** Colloidal particles are charged electrically either positively or negatively. The medium around the particle is charged oppositely in sign, and thus an electric double layer is formed. The charge in the medium is due to an excess of ions of the opposite sign from that of the electric charge on the particle. The charge may be due to adsorption of ions or dissociation of ions. When the charge on lyophobic colloids is neutralized, precipitation takes place.

Burton: The Helmholtz Double Layer Related to Ions and Charged Particles, Colloid Symposium Monograph 4:132 (1926).

McClendon: On the thickness of the Helmholtz double layer, Science 66:200 (1927).

Perrin: Mécanisme de l'électrisation de contact et solutions colloïdales, J. chim. phys. 2:601 (1904).

**Electrophoresis (Cataphoresis).** The magnitude and sign of the charge on the particle may be measured by electrophoresis. The rate of migration of the particle in a given potential gradient varies with the density of the charge on its surface. There has been disagreement as to the location of the surface, but the slip-plane of the particle moving through the medium must bisect the effective electric double layer. In case of extreme hydration of the particle the slip-plane is displaced outward (water is moved along with the particle).

If the colloid is in the form of a sieve or filter, water runs through it when placed in an electrical potential gradient, a phenomenon known as electroendosmose. Electroendosmose may be a factor in glandular secretion.

Kruyt, Roodvoets, and Willigen: Cataphoresis, Electric Charge, Critical Potential and Stability of Colloids, Colloid Symposium Monograph 4:304 (1926).

**The isoelectric point** is the reaction of the solution (H ion concentration) at which no cataphoresis occurs. All sols and gels and ampholytes have isoelectric points. Lyophobic colloids precipitate at their isoelectric points, and lyophilic colloids show the least tendency to stay in solution when at their isoelectric points.

Proteins are composed of amino acids which are ampholytes (both acids and bases) due to the presence of carboxyl and amino groups. Just as in the case of buffers, the dissociation of amino acids is determined by the  $pH$ . When the acid dissociation equals the alkaline dissociation, the amino acid is said to be at the isoelectric point. The following table gives the logarithm of the dissociation constants as acid ( $K_a$ ) and base ( $K_b$ ) as well as the  $pH$  at the isoelectric point of a number of amino acids and dipeptides.

$-\log K_a$	Ampholyte	$-\log K_b$	$pH$ of isoelectric point
3.85	Aspartic acid	11.92	3.03
4.39	Glutamic acid	11.82	3.28
4.79	<i>m</i> -Aminobenzoic acid	10.91	3.79
4.92	<i>p</i> -Aminobenzoic acid	11.63	4.20
7.74	Glycylglycine	10.70	5.52
7.74	Alanylglycine	10.70	5.52
7.82	Leucylglycine	10.52	5.66
8.28	Asparagine	11.74	5.27
8.40	Tyrosine	11.58	5.41
8.66	Phenylalanine	11.89	5.35
8.66	Histidine	8.24	7.21
9.70	Alanine	11.28	6.72
9.74	Glycine	11.55	6.58
9.74	Leucine	11.49	6.54
11.00	Lysine	7.00	9.00
13.96	Arginine	7.00	10.48

Consider glycine for example. If  $HCl$  is added to its solution some glycine hydrochloride may be formed; but not all of the glycine will take part in this process. An  $H$  ion will combine with the amino group forming a glycine cation; and, if an electric current is passed through the solution, the glycine will migrate to the cathode (cataphoresis). If, on the other hand,  $KOH$  is added to the glycine solution, the  $K$  salt is formed, which partially dissociates forming glycine anions. If an electric current is passed through the solution, the glycine will migrate to the anode.

At a certain  $H$  ion concentration, either the glycine will not migrate in an electric field or half will go to the cathode and half to the anode. This is the isoelectric point.

Pauli made the following generalization in regard to ampholytes: When  $K_a = K_b$ , the isoelectric point will be reached in a neutral solution. If the product of  $K_a$  and  $K_b = 10^{-12}$ , at the isoelectric point, there are no neutral molecules but equal numbers of cations and anions. If this product is  $10^{-14}$ , there are a small number of

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neutral molecules at the isoelectric point. If this product is  $10^{-16}$ , there are a large number of neutral molecules at the isoelectric point. If this product is  $10^{-23}$ , all the molecules of the ampholyte are in the form of neutral molecules at the isoelectric point.

Since proteins are composed of amino acids and have some free amino and carboxyl groups, they may behave to some extent like ampholytes.

The isoelectric points of some proteins are given in the following table:

PROTEIN	pH AT ISOELECTRIC POINT	PROTEIN	pH AT ISOELECTRIC POINT
Egg albumin.....	4.8	Oxyhemoglobin.....	6.74
Serum albumin.....	4.7	Pancreas nucleoprotein	
Serum globulin.....	5.4	(trypsin).....	3.52
Casein.....	4.7	From typhus bacilli.....	4.4
Gliadin.....	9.3	From paratyphus bacilli....	4.0
Edestin.....	7.0	Fibrinogen.....	5.0

Ordinary gelatin is isoelectric at  $pH = 4.7$ , but NaCl may displace it to  $pH = 3.3$ .

Hydrophile colloids, when dry, have a strong affinity for water. The Egyptians used such substances in place of dynamite, filling holes drilled in rocks with dry colloidal substances, then wetting them, and allowing the swelling process to break open the rock.

The swelling of colloids immersed in water is influenced by electrolytes. The swelling of polysaccharides is reduced by electrolytes.

Proteins swell least at the isoelectric point, and from this point the first addition of acids or alkalies increases swelling; but salts reduce swelling. Since proteins in the body are on the alkaline side of the isoelectric point, the first addition of acid may reduce swelling.

The precipitation of heat-coagulated protein is most rapid at the isoelectric point.

Lyophilic colloids are said to exert osmotic pressure which is lowest at the isoelectric point. Their viscosity is least at the isoelectric point.

Hitchcock: The isoelectric point of gelatine at  $40^{\circ}$ , J. Gen. Physiol., 6:457 (1924).

**Hofmeister Series.** Lyophilic colloids which usually do not precipitate on neutralization of their electric charges may be

salted-out by large additions of neutral salts. Since gases, and other non-electrolytes such as alcohol, may be salted-out in the same manner, the salt is said to affect the medium. Series of ions used in salting-out are therefore called lyotropic series. Since such series were studied by Hofmeister, they are often called Hofmeister series. For example, Hofmeister determined the molal concentration of sodium salts that would salt-out an albumin sol as follows:

Citrate.....	0.56	Nitrate.....	5.42
Tartrate.....	0.78	Chlorate.....	5.52
Sulfate.....	0.80	Iodide.....	Ineffective
Acetate.....	1.69	Thiocyanate.....	Ineffective
Chloride.....	3.62		

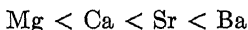
If the albumin solution is made acid, the series is reversed, iodide and thiocyanate being the most effective in salting-out.

The swelling of gels is influenced by the Hofmeister series, precipitating ions causing gels to shrink.

Besides the anion series the cation series is as follows in precipitating fibrinogen:



Divalent ions are much more effective precipitants of lyophobic colloids and form a series for lyophilic colloids as follows:



Kunitz: Valency and alleged Hofmeister series in the colloidal behavior of proteins, III., J. Gen. Physiol. 6:547 (1924).

Neergaard: The reversal of the Hofmeister ion series during volume swelling of colloid mixtures in the form of powder, Kolloid-Z. 35:111 (1924).

**Ultra-filter.** A colloid gel in the form of a membrane acts as an ultra-filter, holding back colloids and in some cases dissolved substances. For example, a freshly precipitated copper ferrocyanide membrane will hold back sugars and  $\text{MgSO}_4$ . "Cellophane" or "visking" (cellulose hydrate) swells with water and then holds back colloids only. The permeability of collodion membranes depends on the degree of drying before immersion in water; if they are dried a long time they may be impermeable to  $\text{MgSO}_4$ . Visking sausage casings are the most convenient form of ultra-filter.

The alimentary canal is lined by a colloid membrane of limited

permeability, or ultra-filter, and hence the colloids from the bodies of animals or plants that are eaten are not absorbed readily. They are, however, hydrolyzed in the alimentary canal and the "building stones" absorbed rapidly.

The glomerulus of the kidney acts essentially as a colloid membrane through which the easily diffusible substances of the blood are filtered out into the urine. A filter with such fine pores that it holds back all the colloid substance is called an ultra-filter. In order to produce ultra-filtration, enough pressure has to be applied to concentrate the colloids. These colloids exert a pressure which we may call osmotic pressure although there is some difference of opinion about its nature. The pressure of the blood against the colloid membrane of the kidney is produced by the contraction of the heart; and, when it exceeds the osmotic pressure of the colloids, urine is secreted. The osmotic pressure of the colloids in the blood causes absorption of the intestinal contents after the food colloids are reduced to molecules of diffusible size.

The pressing out of all the crystalloid elements of the blood into the urine would cause a great loss of nutriment, but this is avoided in the healthy kidney by re-absorption of sugar, amino acids, and those salts that are needed by the body. In case there is not an over-supply of water in the body, most of the water of the urine is re-absorbed, and thus the urine is concentrated.

When the kidney is damaged (nephritis) two things may happen. One is that a great deal of the ultra-filter surface may be put out of action, causing an accumulation of excretory products in the blood; and the other is that the remaining part of the filter surface may be increased in permeability, allowing colloids to escape into the urine (albuminuria).

In nephrosis there is albumin in the urine and usually edema, but no increase in non-protein nitrogen of the blood. The serum proteins are reduced in amount. Possibly the edema is due to low glomerular blood pressure thus reducing the ultra-filtration.

Bechhold: Durchlässigkeit von Ultrafiltern, Z. physik. Chem. 64:328 (1908).

**Anomalous Osmose.** Membranes whose pore diameters are between  $0.1$  and  $0.4\mu$  and whose walls are electrically charged may be the seat of electromotive force and hence of electroendosmose. With a negative membrane, if the negative ion diffuses more rapidly than the positive ion the dilute side will be negative

and drag the positively charged water by electroendosmose to the dilute side. This may be a factor in secretion.

Anomalous osmose has been used in attempts to explain absorption and secretion in the body.

Bartell: Membrane Potentials and Their Relation to Anomalous Osmose, Colloid Symposium Monograph 1:120 (1923).

**Osmotic Pressure.** Membranes with pore diameters of  $0.1\mu$  or less allow the passage of water but inhibit the passage of solutes more or less and hence may be used to measure osmotic pressure. Van't Hoff showed that the formula for gas pressure:

$$PV = RT$$

holds for osmotic pressure, and hence one gram-molecule of non-electrolyte in a liter of water should exert 22.4 atmospheres of osmotic pressure. It usually exerts more pressure than this because the molecules are larger than those in an ideal gas. The size of the molecules may be increased by hydration.

Traube showed that colloid membranes of copper ferrocyanide have small enough pores to be used in osmotic experiments, and Pfeffer and DeVries used porous clay cups impregnated with copper ferrocyanide for measuring osmotic pressure. H. N. Morse made very accurate measurements of osmotic pressure of sugar solutions by this means. These membranes may be used for measuring osmotic pressure of  $MgSO_4$ . KCl solution causes the copper ferrocyanide to crystallize and thus enlarge the pore diameters.

On account of the difficulty of preventing leaks in apparatus for direct measurement, osmotic pressure is usually calculated from the freezing-point lowering of water ( $\Delta$ ) from the equation:

$$P = 12.06 \Delta - 0.021 \Delta^2$$

## DIVISION 2

### CATALYSIS

**Fermentation** was observed by the ancients. It was described in the words of Van Helmont, 1648, by his pupil, Sylvius (1614-72), and also by Vieussens, 1688, as follows:

“ Fermentation is the adventitious and expansive movement of

heterogeneous parts and of insensible fermenting bodies excited without sensible cause, which, when it is vehement or of long duration, brings about an essential change or a conspicuous alteration in the fermenting bodies themselves."

Fermentation could not in those days be distinguished from other chemical processes because ferments had not been isolated. There was a long discussion as to the acidity, neutrality, or alkalinity of fermentations, particularly in the alimentary tract, and digestive juices were collected. Regner de Graaf, student of Sylvius, 1664, placed the quill of a wild duck in the pancreatic duct of a dog and collected pancreatic juice in a flask, obtaining an ounce from a large dog in seven or eight hours. He also inserted a quill into the parotid duct and obtained its digestive juices. He collected human pancreatic juice immediately after death.

No one had collected pure gastric juice, but Van Helmont considered the stomach acid. Brunner, 1682, removed all of the pancreas, except its head, from a dog and kept it alive for a considerable time. He described Brunner's glands in the duodenum and spoke of them as being a secondary pancreas and functioning when the other pancreas was removed. Colored vegetable juices, such as litmus, were introduced as tests for acidity or alkalinity, but much confusion existed as to the stomach, owing to the fact that various animals were worked on and the paunches of ruminants and gizzards of birds were confused with stomachs of other vertebrates.

Haller, 1736, knew that bile dissolved fat and that animals from which the pancreas had been removed were usually hungry (now known to be a symptom of diabetes). Réaumur obtained juice from a kite which swallowed a perforated capsule containing a sponge and then vomited it. By squeezing the sponge he obtained the juice which, when incubated at 32° R. for 24 hours, digested meat. Spallanzani, 1768, swallowed perforated wooden tubes containing meat and found the meat digested when they were voided, thus excluding mechanical action. He vomited from an empty stomach, and incubated meat with the juice by placing it under his arm-pit, noting the digestion and distinguishing this kind of ferment action from that going on outside the body, and subdividing it into vinous, acetous, and putrid.

Scopoli observed salammoniac in the stomach, but it remained for Prout, in 1824, to discover hydrochloric acid in the gastric juice.

William Beaumont, the grand old man of American physiology, began the study of stomach digestion through a fistula in Alexis St. Martin's stomach in 1822 and observed the flow of the gastric juice and the time that the food remained in the stomach.

Although Latour, 1838, showed that alcoholic fermentation is due to micro-organisms, this was not thoroughly understood by the public until the work of Pasteur, 1878. Ferments were classified as organized ferments, such as yeast, and unorganized ferments, such as zymase, when Büchner, 1897, pressed zymase out of yeast. Both are killed by boiling. (These latter were called enzymes (= in yeast, Kuhne, 1878), Duclaux ending the names in "ase").

Dubrunfaut, 1822, obtained a water solution of malt diastase with which he digested starch. Payen and Persoz, in 1833, precipitated diastase from solution by means of alcohol (Liebig and Wöhler precipitated emulsin with alcohol, 1837).

Eberle showed that there was some substance in the neutral mucus of the stomach which became active on treatment with hydrochloric acid, and in 1835 Schwann precipitated pepsin from the gastric juice. In 1836 Purkinje and Pappenheim observed the tryptic action of pancreatic juice.

In 1843 Berzelius described the action of platinum black (discovered previously) of oxidizing alcohol to aldehyde as *catalysis* (contact catalysis). Berthelot observed pancreatic diastase and lipase action. Berzelius gave the name to ptyalin (1881), which had been studied by Leuchs.

Much discussion was waged over the question of why the digestive organs do not digest themselves. This question was probably answered in respect to the stomach by Eberle, who showed that a pro-enzyme was secreted, later called pepsinogen, and by Claude Bernard, who showed by injecting Prussian blue intravenously that the hydrochloric acid appears only at the mouths of the gastric glands (1843). The pancreatic protease is secreted as poorly active trypsin, and this is combined with enterokinase in the intestine to form a more active enzyme.

Considerable work has been done on the rate of enzyme action, and it is often found that the rate of enzyme hydrolysis about doubles with a rise of  $10^{\circ}$ . The rate depends also on the concentration of the substrate, the concentration of the enzyme, the pH, and in many cases the presence of calcium salts ( $\text{Cl}^-$  for amylpsin).

The rate of enzyme hydrolysis follows, to a certain extent, the *law of mass action*. This law implies that the enzyme hydrolysis is *reversible*. Kastle and Loevenhart, 1900, showed that lipase (esterase) action is reversible, at least so far as ethyl butyrate is concerned. Alonzo Taylor, 1908, produced the synthesis of a protein by means of an enzyme (reported by Danielewski, 1896); but, although Robertson and Wasteneys have studied this question considerably, it has never been shown that the action was truly reversible, that is, that the protein obtained by synthesis was the same as the one split by hydrolysis.

Since enzymes speed up the rate of chemical reactions, a clear idea of rates (and their graphic representation) is important.

Nord and Weidenhagen: *Ergebnisse der Enzymforschung*, Leipzig (1932).

**Cartesian Coordinates.** Rectangular Cartesian coordinates are related to two axes perpendicular to each other, one, the axis of  $x$  or axis of abscissas, is horizontal; the other, the axis of  $y$  or axis of ordinates, is vertical.

Rates are measured on a curve. Consider, for instance, the rate at which a body moves through space. If the minutes are plotted on the  $x$ -axis, a straight line will result only if the body is moving at a uniform rate; but if it is a changing rate, as in falling, a curved line will result. The rate at any point in the curve is determined by the angle that the tangent makes with the base line.

**The Law of Mass Action.** According to the law of mass action, the rate of a chemical reaction depends on the concentration of the chemical substance in the reactive form. Take, for example, the inversion of cane sugar. The rate of the inversion is proportional to the concentration of the cane sugar. If not all the cane sugar is in the active form, at least a constant fraction of it is, and inversion is still proportional to sugar concentration. In the inversion of cane sugar, let  $x$  equal the cane sugar hydrolyzed in mols per liter of solution in the time,  $t$  and  $\frac{dx}{dt}$  the rate of inversion. Now, let the *initial concentration* of cane sugar be 1 mol per liter, and it will therefore be  $(1 - x)$  at any later time. The rate of reaction may be expressed algebraically as follows:

$$\frac{dx}{dt} = k(1 - x)$$

In this equation  $(1 - x)$  represents the concentration of cane sugar and  $k$  is a constant (velocity constant).

Reactions may proceed simultaneously in opposite directions (reversible reactions). In the hydrolysis of ethyl butyrate, for instance, if  $x$  equals the concentration of alcohol (being equal to that of butyric acid) and  $(1 - x)$  equals the concentration of ethyl butyrate,

$$\frac{dx}{dt} = k_1(1 - x)$$

The reaction will proceed simultaneously in the opposite way (is reversible), and so another equation may be written:

$$\frac{dx}{dt} = k_2(\text{alcohol}) \times (\text{butyric acid}) = k_2x^2$$

At equilibrium, ethyl butyrate is being synthesized at the same rate it is being hydrolyzed. The reaction proceeds in both directions at the same speed, hence we may combine the two equations:

$$k_2x^2 = k_1(1 - x)$$

Simplifying this equation we obtain

$$\frac{x^2}{1 - x} = \frac{k_1}{k_2} = K$$

$K$  is called the equilibrium constant.

**Enzymes** are catalysts, and for that reason they cannot change the equilibrium point of a chemical reaction. In case they combine with the end-products of a reaction and change the equilibrium point that way, then they are not true catalysts. True enzymes affect only the rate of a reaction. Temperature changes may affect the equilibrium point and speed of reaction; salts influence speed; H ions affect the speed of action of enzymes.

Since the rate of the reverse reaction increases as the equilibrium point is approached, the rate of hydrolysis with enzymes will diminish with time. The Schütz-Borissov formula is

$$X = K(aet)^{1/2}$$

$X$  is the substrate hydrolyzed,  $t$  is the time,  $a$  is the initial concentration of the substrate, and  $e$  is the concentration of the enzyme. Northrop found that this formula would not hold in all cases but only in special ones, for instance, in the hydrolysis of protein by pepsin, in which there is a combination of peptone with the pepsin. Schütz' rule, however, expresses the rate more closely than any other simple equation.

In some instances the rate is influenced by destruction of the enzyme. Most enzymes exhibit maximum activity between 37° and 53°. Higher temperatures may produce some destruction. Some enzymes show their highest activity at 80–85° (malt diastase first isolated by Payen and Persoz). It is probable that all enzymes show increased rate of activity with a rise of temperature but also an increased destruction. Many enzymes show inactivation beginning at about 50°. Invertase is protected by the presence of the substrate so that it will stand temperatures 20°

higher. Trypsin is destroyed rapidly by boiling in an alkaline solution but only slowly in an acid solution. Invertase, trypsin, and taka-diastrase, inactivated by boiling, may to a certain extent, be reactivated.

Payen and Persoz: *Ann. chim. phys.* 53:73 (1833).

**Specificity.** Berzelius' idea of an enzyme as a catalyst taking no part in the reaction implies that it merely speeds a reaction that would go on without it, or overcomes a resistance which normally causes the reaction to go on at a very slow or perhaps zero rate, and therefore it cannot change the equilibrium point of a reaction. In a reversible reaction there would be both quantitative and qualitative reversibility. It was shown, however, by Croft-Hill (1898) that, although maltose treated with yeast maltase yields glucose, maltase acting on a concentrated solution of glucose yields isomaltose. This does not imply that the reverse reaction takes a different course but that if one starts with either maltose or isomaltose and adds maltase, glucose will be formed, but if one starts with a concentrated solution of glucose and adds maltase, both maltose and isomaltose will be formed in a definite ratio. Perhaps this would be considered the effect of two enzymes in the yeast maltase, one affecting isomaltose and the other maltose. Maltase of yeast acts on  $\alpha$ -methylglucoside and emulsin on  $\beta$ -methylglucoside. If a mixture of the two glucosides is incubated with yeast the  $\alpha$  form is split and fermented, leaving the  $\beta$  form.

An enzyme is supposed to affect only one linkage — thus, if the trisaccharide, raffinose, which is formed of three sugar molecules (with therefore two bonds between them), is treated with yeast saccharase, bond 1 is broken and a mixture of fructose and melibiose is formed; but if it is treated with emulsin from bitter almonds, bond 2 is broken and a mixture of sucrose and galactose is formed.

It is claimed by Sumner that urease is a crystalline protein and by Northrop that trypsin and pepsin are crystalline proteins. Sumner found that crystalline urease required a protector to preserve its activity when exposed to copper in distilled water.

Waldschmidt-Leitz describes an enzyme as a chemical group usually containing asymmetric carbon atoms and hence of a particular stereochemical form that is attached in some way to a carrier which is a colloidal substance and which might act as a

protector. In every case the enzyme has been shown to contain protein but it is not known whether the protein is the carrier or the active group. Whether the carrier can ever vary without affecting the enzyme action has not been shown, but change of carrier has varied enzyme action in certain cases. It is not known whether the stable enzyme body is a chemical compound in the ordinary sense or a colloidal complex.

**Co-Enzyme.** The active enzyme solution has been shown in many cases to be composed of two bodies, one being thermolabile and usually termed the enzyme proper and the other thermostable and usually termed co-enzyme. Some co-enzymes are of known chemical composition, such as phosphates in relation to ptyalin and bile salts in relation to lipase.

After Büchner separated zymase from yeast cells by grinding them up with sand and subjecting them to pressure, Harden and Young found that it could be separated into two fractions, an inactive colloid, apozymase, and an inactive (thermostable, dialyzable) filtrate, the co-enzyme. The former was activated when treated with the latter. Recent work has pointed to the presence of phosphates, adenosine polyphosphoric acid, and probably magnesium salts as essential constituents of the co-enzyme, cozymase.

Enterokinase is not considered a co-enzyme although it combines with "trypsinogen" to form active trypsin (or in the language of Waldschmidt-Leitz trypsin combines with enterokinase to form trypsinkinase) since it is thermolabile and hence may be considered an enzyme itself.

**Inhibitors and Inactivators.** Anti-enzymes have been described. In some cases these are supposed to unite with the enzyme, thus preventing its action on the substrate proper. Some of these anti-enzymes are closely related to the substrate but are more refractory, and the enzyme may be considered as being engaged in the hydrolysis of an anti-enzyme and thus being diverted from action on the substrate.

Salts of heavy metals are very injurious to enzymes. The presence of the substrate may make them less injurious. Mercury, silver, lead, and arsenic salts are particularly injurious. If Nessler's solution (containing mercury) is introduced into a glass vessel and this is subsequently cleaned in the ordinary manner for chemical glassware and then the vessel is used for urease, the

urease is usually destroyed. It is stated that the mercury may be removed by nitric acid. This acid should be washed out with distilled water since nitric acid acting on mercury forms mercurous nitrate and the addition of chloride in tap water would form mercurous chloride which is very poorly soluble and might be precipitated on the glass. Some workers boil nitric acid in the glass to remove Nessler's solution and others buy new glassware and reserve it for urease alone.

Fluorides inhibit animal lipase. This may be due to their precipitating calcium.

Anesthetics in general inhibit enzyme action, but the quantity of anesthetic necessary to do this is usually larger than that required for anesthesia of the higher nervous centers.

Hydrocyanic acid inhibits catalase and oxidase but also inhibits catalytic action of platinum black, whereas it activates kathepsin and papain.

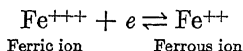
**Phosphate and Zymase.** Although the enzyme of yeast concerned in alcoholic fermentation is called zymase, there are a host of enzymes in yeast and several are concerned in the rapid transformation of sugar into alcohol and  $\text{CO}_2$ . Phosphate greatly accelerates the action of yeast-juice on sugar but the rapid fermentation soon slows down and is increased again by further addition of phosphate. Apparently it is the enol form common to glucose, fructose, and mannose that is fermented. Phosphate may increase the enolization. Since the enol form is unstable, the action of phosphate is transitory. Fructose ferments more readily than glucose probably because it more easily changes to the enol form.

**Proteolytic enzymes** are divided into four groups: (1) pepsin, (2) kathepsin, (3) trypsin, (4) erepsin. Pepsin acts best in acid (pH of 2), trypsin in slightly alkaline solution. The second group includes kathepsin and papain and acts best at pH 4-7 and is activated by HCN,  $-\text{SH}$  compounds, and heavy-metal complexes. Erepsin is subdivided into amino-polypeptidases, carboxy-polypeptidases, and dipeptidases. More details about enzymes are given under the substrates.

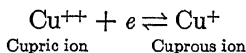
Bayliss: *The Nature of Enzyme Action*, fourth edition, Longmans, Green & Co., New York (1919).

Falk: *The Chemistry of Enzyme Actions*, second edition, Chemical Catalog Co., New York (1924).

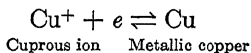
Oxidation-reduction of iron may be expressed as:



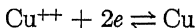
in which  $e$  represents a negative electron; and that of copper:



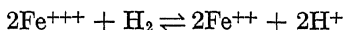
This is a comparable reaction to:



which takes place when a piece of metallic copper is placed in a solution of cuprous chloride. In a solution of cupric chloride, the reaction is:



The tendency of this reaction to run from right to left (G. N. Lewis) is called the electrolytic solution tension of copper and in a normal solution of cupric ions is 0.345 volt measured against the hydrogen electrode as a standard. In normal cuprous ions it is 0.522 volt and in any mixture of cuprous and cupric ions of an intermediate value. In this case,  $\text{Cu}^{++}$  is called the oxidant (Ox) and  $\text{Cu}^{+}$  the reductant (Red). Thus, the oxidation-reduction potential (in volts) depends on the ratio of oxidant to reductant. The oxidation-reduction potential depends not only on the ratio of oxidant to reductant, but also on the hydrogen-ion concentration as can be expressed by the following scheme:



Such oxidation-reduction reactions are reversible, but oxidation with atmospheric oxygen in the absence of metallic electrodes requires a catalyst to break down the resistance of  $\text{O}_2$  to decomposition. Iron and copper are often used for such catalysis, and it is thought that iron in organic combination as heme compounds in living cells is necessary for oxidations.

Wieland supposes that the first step in physiological oxidations is dehydrogenation, the hydrogen uniting with an *acceptor*.

Hopkins isolated a hydrogen acceptor, glutathione, which acts by transformation of an  $-\text{S}-\text{S}-$  group into 2  $-\text{SH}$  groups.

Thunberg has shown that succinic acid is dehydrogenated by living tissues and during the reaction will reduce methylene blue

to the leuco compound. This takes place by means of an enzyme called oxidase or dehydrogenase.

In the presence of  $O_2$  and heme (iron) compounds, glutathione is changed back to the  $-S-S-$  form and heat is liberated.

Kolthoff: *pH and Electrometric Titrations*, John Wiley & Sons, New York (1931).

## DIVISION 3

### CALORIMETRY

The heat evolved during life depends on the principle of the conservation of energy, which is the first law of thermodynamics. Thermodynamics was worked out from the study of the steam engine and applied later to energy changes occurring in our bodies. Chemical reactions are all accompanied by evolution or absorption of heat. Those accompanied by the evolution of heat are called exothermic. The study of energy in terms of heat is called calorimetry.

The reactions of living matter are largely of two types: (1) oxidation and reduction accompanied by large heat changes, and (2) hydrolysis and synthesis with the loss or gain of water and accompanied by slight changes in heat.

The heat,  $q$ , evolved by an exothermic reaction is equivalent to the sum of free and bound energy. Only part of the total energy may be harnessed by a machine to do useful work,  $w$ , and that amount is called the free energy. From any combustion in the body no more than the free energy of these reactions may be transformed into work. The rest of it is lost or used to maintain the temperature of the body.

The fraction available for work is  $w/q$  and is usually rather small. These molecules in order to react one with the other must be in certain relative positions. Only a small percentage are in the right position and the others are not in the correct position and so do not take part in a reaction resulting in useful work. In other words, thermodynamics is based on statistics, but the large number of molecules involved makes calculation very accurate. The maximum amount of muscular energy that might be obtained from one gram of glucose, for instance, may be determined from thermodynamics. Perfect machines for the transformation of chemical into mechanical energy deliver the total energy only at the equilibrium point of a reversible reaction.

Atwater and Benedict: *Experiments on the metabolism of matter and energy in the human body*, U. S. Dept. Agr., Bull. 69 (1899); 109 (1902); 136 (1903).

Lavoisier: *Expériences sur la respiration des animaux et sur les changements qui arrivent à l'air en passant par leurs poumons*, Mem. de l'Acad., 185 (1777).

Priestley: *Experiments and observations on different kinds of air*, second edition.

Rubner: *Die Quelle der tierischen Wärme*, Z. Biol., 30:73 (1894).

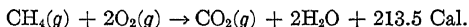
**Heat of combustion of foodstuffs.** The heat evolved by the body is the algebraic sum of the heat of exothermic and endothermic reactions. The unit used in practice is the kilogram calorie, in this book abbreviated Cal., which is the heat required to raise one kilogram of pure water from 15° to 16° C.

In order to find the heat of combustion of a chemical compound, the heat of formation of the substance in question must be deducted from the heat of combustion of the elements. The state — gas (*g*), liquid (*l*), or solid (*s*) — must be defined, since in passing from one physical state to another energy changes are involved. These changes, however, are slight compared with the heat of combustion or the heat of formation.

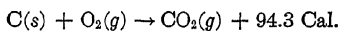
The heat of combustion of any compound is not the same as the heat of combustion of the elements in it, but there is a difference which is equal to the heat of formation of that compound from the elements. For instance,



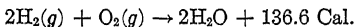
In this reaction a certain amount of heat is given out which might be determined directly if the reaction could be made to take place. It may also be determined indirectly. For instance, consider the heat of combustion of methane:



The heats of combustion of the elements, carbon (and oxygen) and hydrogen (and oxygen), are known.



and



The sum of these two equals 230.9, and subtracting 213.5 gives 17.4 Cal., which is the heat of formation of methane and agrees fairly well with the value given above.

In that way the heat of formation of all the sugars, proteins, and fats may be calculated roughly from the heat of combustion of these substances.

The heat of combustion of the elements of  $\text{C}_6\text{H}_{12}\text{O}_6$  is  $6 \times 94.3 = 565.8 + (3 \times 136.6)$ , giving a total of 975.6. But the heat of combustion of glucose determined experimentally is 677.2. The difference of 298.4 Cal. is the heat of formation of glucose, within reasonable limits of error.

The heats of combustion of foodstuffs have been determined directly, and since the molecular weight of some of them, for instance, proteins, is not accurately known, the value of the heat of combustion per gram is given.

The heat of combustion of the hexose monosaccharides is about 3.75 per gram; of the disaccharides, about 3.95. The difference is due to the fact that in the synthesis of disaccharides from monosaccharides one molecule of water is taken out. So the heat of combustion per gram is greater because a small fraction of water is taken out of the molecule, thus reducing the weight. The heat of combustion of starch is still greater because a greater amount of water is removed in synthesis. Rubner accepted the average value: 4.1 Cal. per g. for mixed carbohydrates.

## KILOGRAM CALORIES

(These heat values are all positive)

	ecular ight	Heat of for- mation per mol (s)	Heat of com- bustion per mol (s)	Heat of com bustion per gram
Glucose.....	180	302.6	677.2	3.76
Fructose.....	180	302.1	675.9	3.75
Sucrose.....	342	535.6	1355.0	3.96
Lactose.....	342	535.6	1351.4	3.95
Dextrin.....	162	243.6	667.2	4.12
Inulin.....	162	231.4	678.3	4.19
Starch.....	162	225.9	684.9	4.23
Stearic acid..	284	222.6	2682.0	9.45
Glycerol.....	92	165.6	397.1	4.32
Glycine.....	75	126.2	234.9	3.13
Alanine.....	89	135.2	389.2	4.37
Ethyl alcohol	46	69.9	325.7	7.08

The heat of combustion of stearic acid is 9.45 Cal. per g. The reason for the high value is that there is very little oxygen in the molecule, and it gives off heat not only in the burning of the carbon but also in the burning of the hydrogen. Stearic acid is approximately  $(\text{CH}_2)_n$ .

In the combustion of the substances listed above, with the exception of glycine and alanine, the end-products are  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , and are the same whether burned inside the body or outside, but where incomplete burning takes place, not all the heat is liberated, and it is necessary to know the calorific value of the end-products in order to calculate the heat of combustion of the original substance in the body tissues. In the living body, protein is not completely burned. So in calculating the heat of combustion of protein, the heat of combustion of urea should be subtracted, urea being made of  $\text{CO}_2$ , which is completely burned, and  $\text{NH}_3$ , which is capable of combustion to  $\text{N}_2\text{O}_5$ .

It has been calculated that in burning body protein, 3.84 Cal. per g. are produced, but in burning meat protein, 4.42 Cal. per g. are produced. Taking the meat without purifying it, the "protein" ( $\text{N} \times 6.25$ ) in it produces 4 Cal. per g. Casein produces 4.4 Cal. and some vegetable protein 3.96. Rubner accepted the average figure for protein of 4.1 Cal. per g., which is the value usually used, calculating protein as  $\text{N} \times 6.25$ .

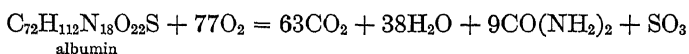
In severe diabetes not all the protein is burned in the body,

but about 58% is converted into sugar and excreted as such, greatly reducing the calorific value of the protein. BANGALORE

Olive oil produces 9.384 Cal. per g., the fat of adipose tissues 9.372 and butterfat 9.179, the lower value for butter fat being due to the presence of lower fatty acids with higher percentages of O<sub>2</sub>. Rubner accepted the average value of 9.3 Cal. per g. for fat. Elimination of ketone bodies in ketosis would lower the calorific value of fats (reducing that of palmitic acid by about  $\frac{1}{4}$ ).

Landolt-Börnstein-Meyerhoffer: Physikalisch-chemische Tabellen, Springer, Berlin, third edition, p. 148 (1905).

**Respiratory quotient** is the ratio of CO<sub>2</sub> to O<sub>2</sub> (either by volume or mols) in the combustion of foodstuff.



$$\text{The respiratory quotient R.Q.} = \frac{\text{mols CO}_2}{\text{mols O}_2} = \frac{63}{77} = 0.818$$

In determining the respiratory quotient the CO<sub>2</sub> and O<sub>2</sub> are measured by volume because equal volumes contain equal numbers of molecules since 1 mol gas = 22.4 liters.

A crude method of determining the R.Q. for protein is to starve a person so that he is not burning any sugar and then give him a large protein meal and determine the heat given out by the body and the nitrogen in the urine, assuming that the nitrogen came from the burning of the protein. The carbon dioxide given out, the oxygen absorbed, and the nitrogen excretion must be determined, and ratios should be correct for protein combustion. If starved, a person would burn body protein, fat, and a small amount of carbohydrate. The protein meal is intended to be so large that the fat or carbohydrate would not be burned.

In the normal metabolism, only protein, carbohydrate, and fat are burned, but in diabetes and also in the process of fattening, other processes are going on that affect respiration, which will be considered later.

The R.Q. for carbohydrate is 1.

Lusk: Science of Nutrition. Fourth edition, Saunders, Philadelphia (1928).

**Specific Dynamic Action of Protein.** Metabolism varies from day to day (fig. 8), and the causes of such changes have been studied. When protein is eaten, the combustion of protein as

well as of other foodstuffs is increased. A large protein meal will increase the resting heat production 50%. This action of protein is called specific dynamic action. Certain amino acids have a specific dynamic action and it seems probable that the specific dynamic action of protein is the sum of the specific dynamic action of the amino acids.

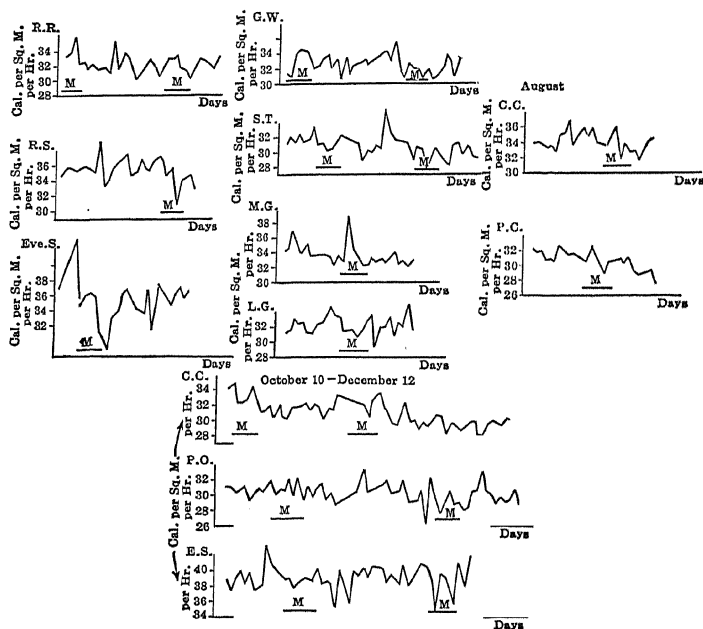


FIG. 8. Daily variations in basal metabolic rate. Archive of Internal Medicine.

Lusk has studied the specific dynamic action of various amino acids and found the greatest effect due to glycine and alanine, but Mann has shown that this is abolished by removal of the liver. Mann concludes that the extra heat is due to deaminization of glycine and alanine and sugar synthesis.

Mann, Wilhelmj and Bollman: *Am. J. Physiol.* 81:496 (1927).

Ort and Bollman: *J. Am. Chem. Soc.* 49:805 (1927).

**Protein Metabolism.** When proteins are burned in the body, one gram of urinary nitrogen corresponds to 6.04 liters of oxygen

and to 4.88 liters of carbon dioxide. In determining the protein metabolism, determine the grams of urinary nitrogen for the period under consideration and multiply that by 4.754 to obtain the carbon dioxide for that period. Multiply the grams nitrogen by 5.91 ( $= 26.51 \div 4.485$ ) to obtain the liters of oxygen. The protein calories are obtained by multiplying the grams of nitrogen by 26.51 (or  $25.6 = 4.1 \times 6.25$ ).

**Non-Protein Metabolism.** Subtract the protein metabolism from the total and the remainder is carbohydrate and fat (non-protein) metabolism.

The separation of non-protein metabolism into carbohydrate and fat is rather complicated but may be simplified by the use of the following chart (Zunz and Schumburg, Lusk). Determine the non-protein R.Q. and read from the table the grams of carbohydrate per liter of oxygen, grams of fat per liter of oxygen, and Calories per liter of oxygen. If the R.Q. is 0.8, for example, 0.375 g. of carbohydrate and 0.350 g. of fat would be burned and 4.801 Cal. would be given out per liter of oxygen.

From chart p. 32 you may see also what the variations would be in guessing the R.Q. if no protein is burned and the oxygen alone determined. With pure carbohydrates, the R.Q. would be 1, since the number of mols of  $O_2$  absorbed and  $CO_2$  eliminated are equal, and 5.047 Cal. per liter of oxygen or carbon dioxide would be given out. If the R.Q. is assumed to be 0.82, 4.825 Cal. per liter of oxygen would be calculated, the error being about 4%. If metabolism is determined from the  $CO_2$  alone, a much greater error would arise since the Calories per liter of  $CO_2$  is 5.047 when carbohydrate is burned and 5.89 when R.Q. = 0.82, or 16.7%. What error might arise from not considering the protein? A person ordinarily burns less than 100 g. protein in 1,000 g. food-stuff, so that error would be small.

Lusk: J. Biol. Chem. 59:41 (1924).

Zunz and Schumburg: Studien zu einer Physiologie des Märsches, Berlin, p. 361 (1901).

**The Bomb-calorimeter.** The instrument in general use for the determination of heat of combustion of organic compounds is called the bomb-calorimeter invented by Marcolin Berthelot. The substance is placed inside a steel bomb with non-corrosive lining, and filled with compressed oxygen and ignited by means of an iron wire heated by an electric current. The lining is made

## NON-PROTEIN METABOLISM

Non-Protein respiratory quotient	One liter of oxygen is equivalent to			Calories per liter CO <sub>2</sub>
	Grams		Calories per liter O <sub>2</sub>	
	Carbohy- drate	Fat		
0.707	0.000	0.502	4.686	6.61
0.71	0.016	0.497	4.690	
0.72	0.055	0.482	4.702	
0.73	0.094	0.465	4.714	
0.74	0.134	0.450	4.727	
0.75	0.173	0.433	4.739	
0.76	0.213	0.417	4.751	
0.77	0.254	0.400	4.764	
0.78	0.294	0.384	4.776	
0.79	0.334	0.368	4.788	
0.80	0.375	0.350	4.801	5.89
0.81	0.415	0.334	4.813	
0.82	0.456	0.317	4.825	
0.83	0.498	0.301	4.837	
0.84	0.539	0.284	4.850	
0.85	0.580	0.267	4.862	
0.86	0.622	0.249	4.875	
0.87	0.666	0.232	4.887	
0.88	0.708	0.215	4.899	
0.89	0.741	0.197	4.911	
0.90	0.793	0.180	4.924	5.047
0.91	0.836	0.162	4.936	
0.92	0.878	0.145	4.948	
0.93	0.922	0.127	4.961	
0.94	0.966	0.109	4.973	
0.95	1.010	0.091	4.985	
0.96	1.053	0.073	4.998	
0.97	1.098	0.055	5.010	
0.98	1.142	0.036	5.022	
0.99	1.185	0.018	5.035	
1.00	1.232	0.000	5.047	
Protein metabo- lism	0.818	0.000	4.485	

of glass-enamel, platinum, chromium-nickel steel, or other alloy. The bomb lies immersed in a weighed quantity of water inside a Dewar flask. The water surrounding the bomb is stirred with a stirrer that produces very little friction (of a known heat equivalent). The mass of water in the calorimeter being known, and its temperature having been read before and after combustion, the quantity of heat evolved may be calculated. Since there are metal parts in the bomb, the heat capacity of the apparatus must

be measured, and the heating effects of the electric current and that due to combustion of the iron (and that due to stirring, if significant) must be subtracted from the total.

Berthelot: *Leçons sur la thermochimie*, Paris (1865).

**Direct Animal Calorimetry.** If mixed foods are burned in the bomb-calorimeter, a higher value is obtained than that from burning in the body because the protein is not completely burned in the body. We may digest and burn starch and disaccharides; but we cannot completely burn pentoses, pentosans, agar, cellulose, lignin, chitin, or related substances. Starch of string beans is not entirely digested because enzymes cannot penetrate the cellulose cells walls. These substances are partially fermented and the fermentation products burned. The part not burned may be determined by burning the feces in a bomb-calorimeter.

Lavoisier used a Laplace calorimeter made by hollowing a block of ice and covering it with another block. The animal was placed inside, and any heat coming from the outside was used in melting the outside of the block; the heat from the animal on the inside was used in melting the inside of the block, and the kilograms of resulting water on the inside measured the Calories because 80 Cal. are absorbed per kilogram of ice melted.

Atwater and Rosa developed a calorimeter which consisted of a little room that had three walls, two of which were metal separated by an air space, and the third was a cork wall on the outside. These two metal walls were connected by thermocouples so that any difference in temperature between the outer and inner metal walls was immediately registered. The outer wall was then heated by electric heaters to the temperature of the inner wall so that no heat would be lost. A cooling coil was installed so that the air would not become too warm inside. The temperature of the water going in and coming out of the coil was measured, and the water weighed, to determine the Calories produced by the person inside the calorimeter.

Calorimeters of this type were used at Wesleyan University (later transferred to Carnegie Nutrition Laboratory, Boston); U. S. Department of Agriculture, Washington; and Russell-Sage Institute, Bellevue Hospital, New York City.

Atwater and Benedict: A respiration calorimeter with appliances for the direct determination of oxygen, Carnegie Inst. Wash., Pub. No. 42 (1905).

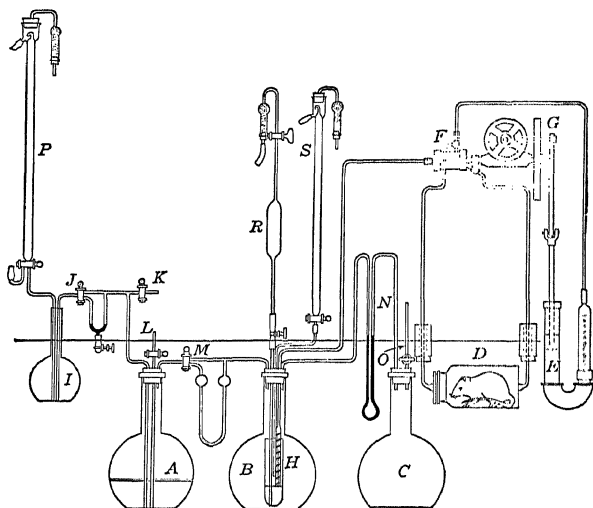


FIG. 9. Apparatus for respiratory quotients and basal metabolism of the rat. L. G. Wesson.

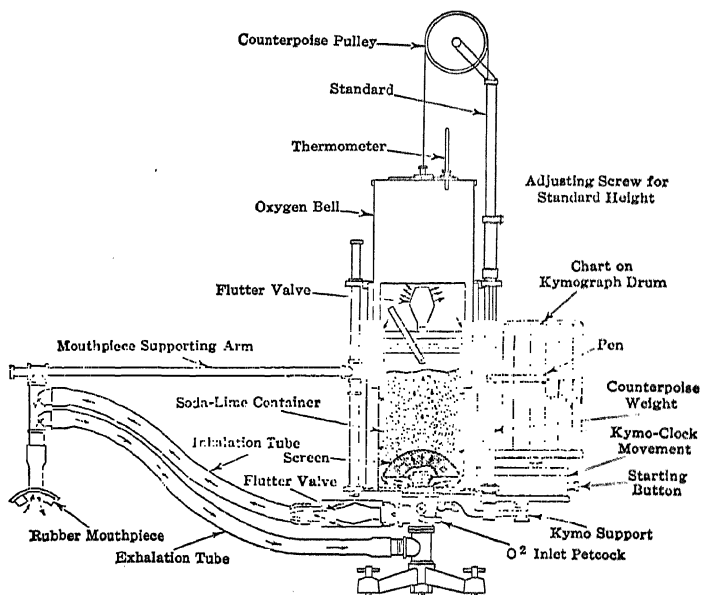


FIG. 10. Benedict-Roth Apparatus. Warren E. Collins.

**Indirect Calorimetry.** Lavoisier used indirect calorimetry in studying how much oxygen a man used. Indirect calorimetry was perfected by Atwater and Benedict. If one determines the oxygen absorbed and  $\text{CO}_2$  and nitrogen eliminated, he may calculate the grams of protein, fat, and carbohydrate burned.

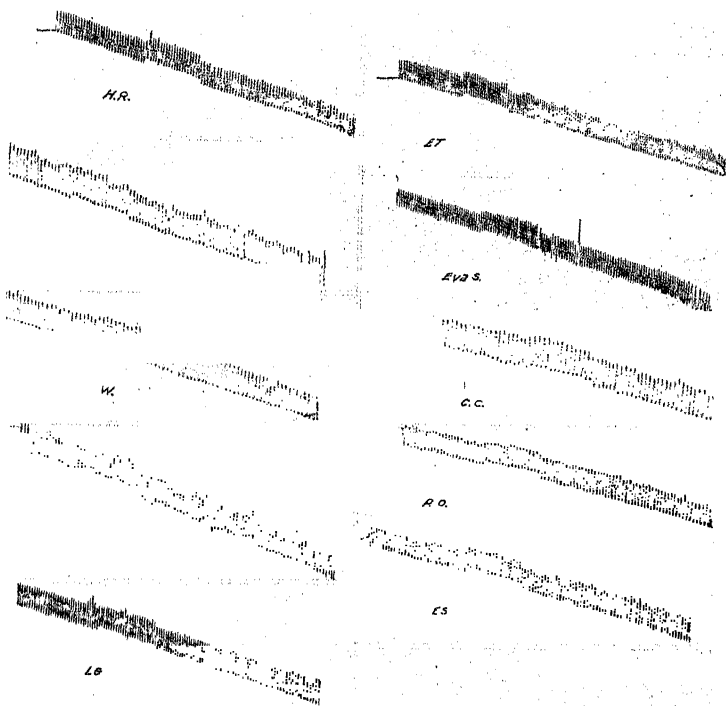


FIG. 11. Tracing on Benedict-Roth apparatus. Archives of Internal Medicine. The jog is due to a weight to test for leaks.

A Haldane apparatus is used to analyze the air, and a Tissot spirometer (or gas meter) is used to measure the air-volume. The exhaled air is blown into the spirometer, measured, and analyzed in the Haldane apparatus. Inspired dry air is about 21%  $\text{O}_2$ ; but, when saturated with moisture at room temperature, it is about 20.5%  $\text{O}_2$  and the  $\text{O}_2$  of expired air should be subtracted from the latter figure to determine the  $\text{O}_2$  absorption. Apparatus

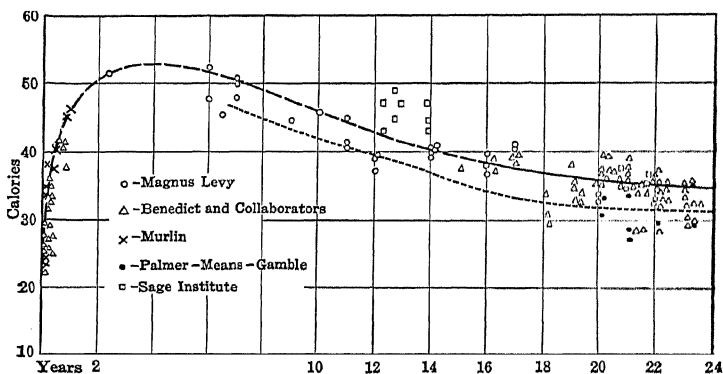
for small animals has been modified by various investigators (fig. 9).

With the portable Benedict apparatus (fig. 10), the carbon dioxide eliminated is not measured. Ordinarily about 0.82 is accepted as the normal respiratory quotient (R.Q.) and therefore 4.825 Cal. per liter of oxygen will be produced (fig. 11).

Benedict and Carpenter: Respiration calorimeters for studying the respiratory exchange and energy transformations of man, Carnegie Inst. Wash., Pub. No. 123 (1910).

Wesson: J. Nutrition, 3:503 (1931).

**Basal Metabolism.** Ordinarily what is known as the basal metabolism, that is the metabolism in rest and fasting, is about 40 Cal. per sq. m. per hour for a 20-39 year old man or a 16 year



[ FIG. 12. DuBois chart of basal metabolism, Cal. per sq. m. hr. and age. Archives of Internal Medicine.

old girl, and then drops with age, it being 37 for a 20-29 year old woman, 39 for a 40-49 year old man, and 36 for a 30-49 year old woman (Aub.). In order to obtain basal conditions, the patient should fast 14 hours (or eat no supper or breakfast) and rest for  $\frac{1}{2}$  hour in bed immediately before the experiment. The subject's own temperature must be normal, since it has been shown that an elevation of body temperature of  $1^{\circ}$  will, on the average, increase the metabolic rate by 10 per cent. Benedict finds the normal skin temperature  $28^{\circ}$  to  $35^{\circ}$ , but on exposure it may go down to  $20^{\circ}$ . The skin temperature for greatest comfort is  $33.5^{\circ}$  on neck and forehead.

Dubois: Basal Metabolism in Health and Disease, Lea & Febiger, Philadelphia (1924).

Heat loss is by radiation and conduction or by evaporation (fig. 13). The total heat loss is nearly constant until shivering occurs. Then it rises with lowering of temperature. The heat

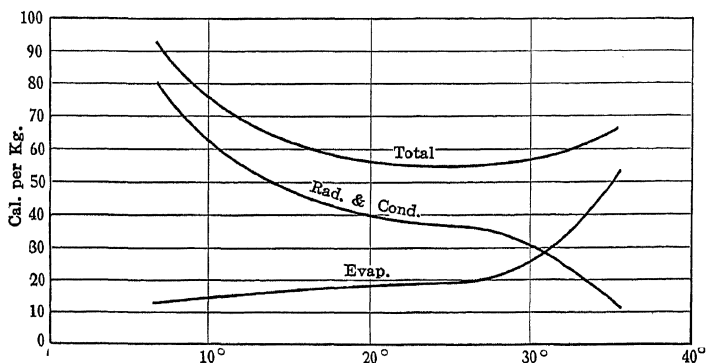


FIG. 13. Heat loss. Archiv für Hygiene.

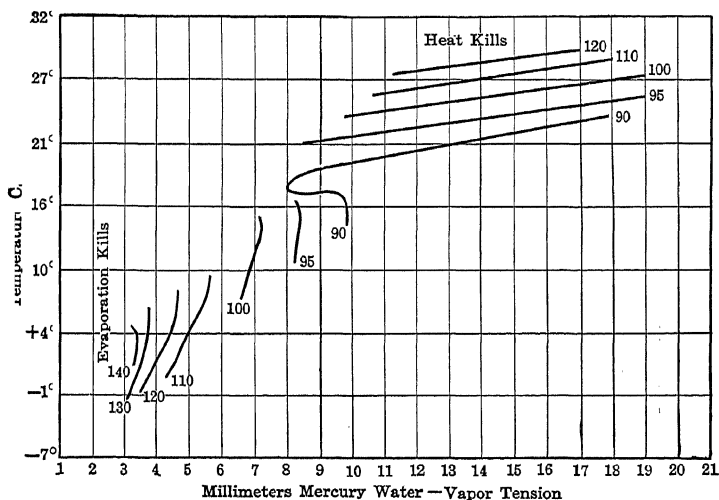


FIG. 14. Huntington's data of climate and death rate, recalculated by Nelson Taylor.

lost by evaporation increases enormously after the air temperature approaches body temperature, and that by radiation and conduction is lowered. If the air is saturated with moisture and evaporation cannot occur, death results if the air temperature

risers to about  $40^{\circ}\text{C}$ . An average external temperature of  $20^{\circ}\text{C}$ . outdoors is most healthful. At low temperatures the cooling effect of dry air is detrimental to health. At high temperatures there is difficulty in dissipating body heat resulting in a high death rate (fig. 14).

Huntington: Weather and Health, National Research Council, Committee on the Atmosphere and Man, Bull. 75.

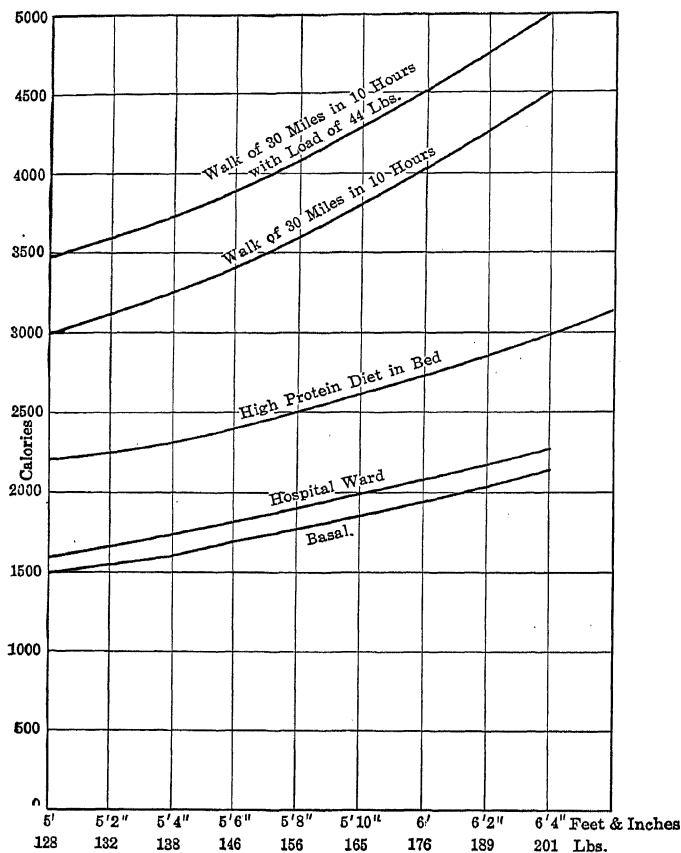


FIG. 15. Metabolism and various activities. Lusk-Zunz.

**Surface Law.** Rubner showed that the basal metabolic rate is proportional to the surface area. This was at first thought to be due to the fact that heat loss is proportional to surface area

(Newton's law of cooling) and to maintain the body temperature constant would require heat production to equal heat loss. But

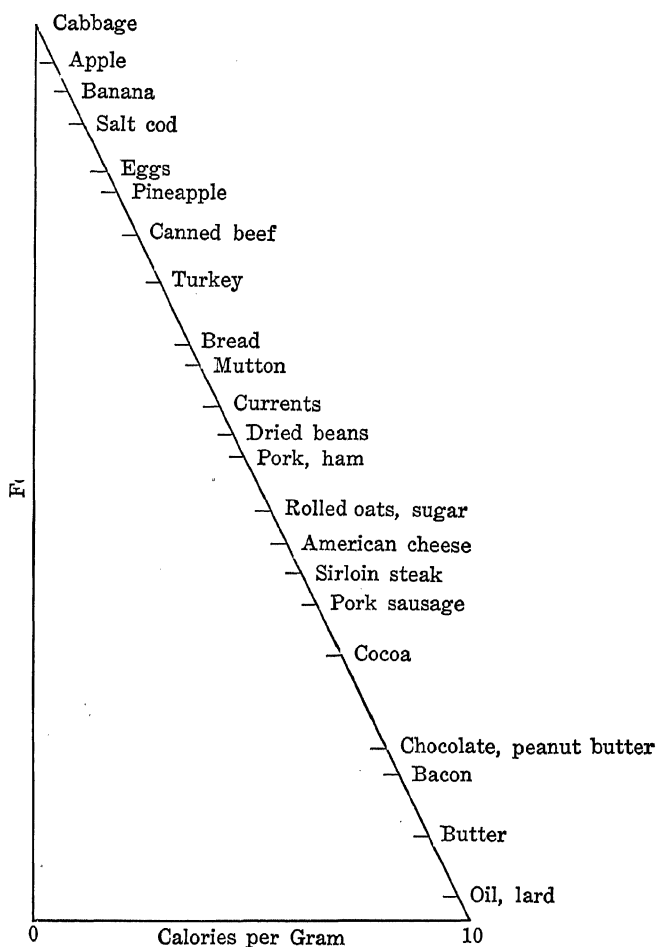
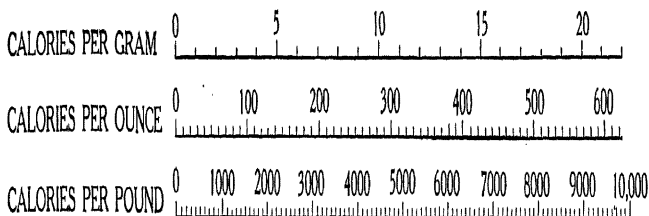


FIG. 16. Food-stuffs arranged according to calorific value.

the surface law seems to hold for cold-blooded animals as well as for those that maintain their body temperature constant.

The surface area of adults may be determined by using Dubois' chart. Scammon's formula is: Square centimeters =  $1,008 \text{ kg}^{0.1692}$ .



## FOOD

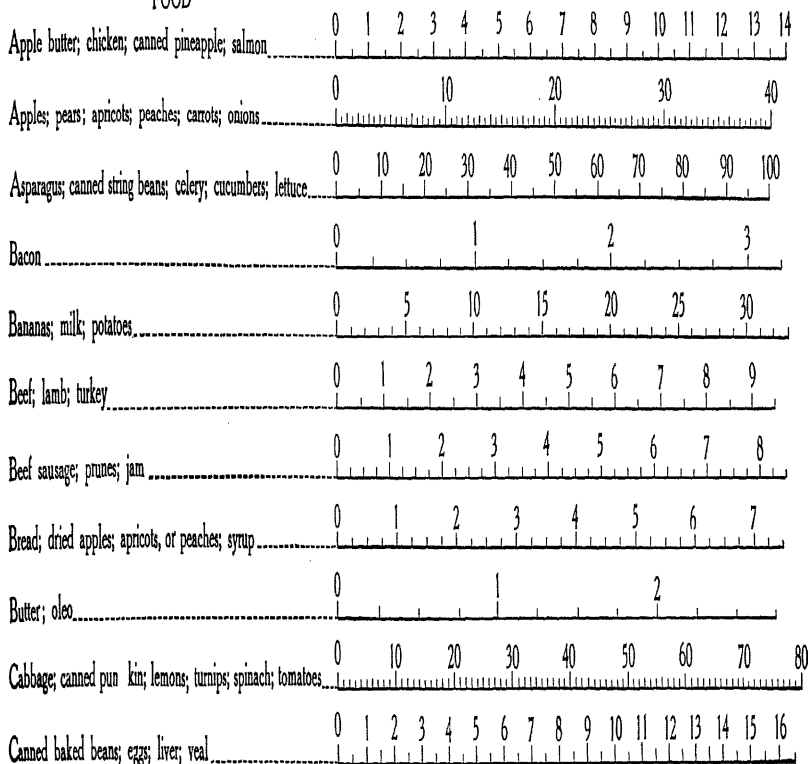
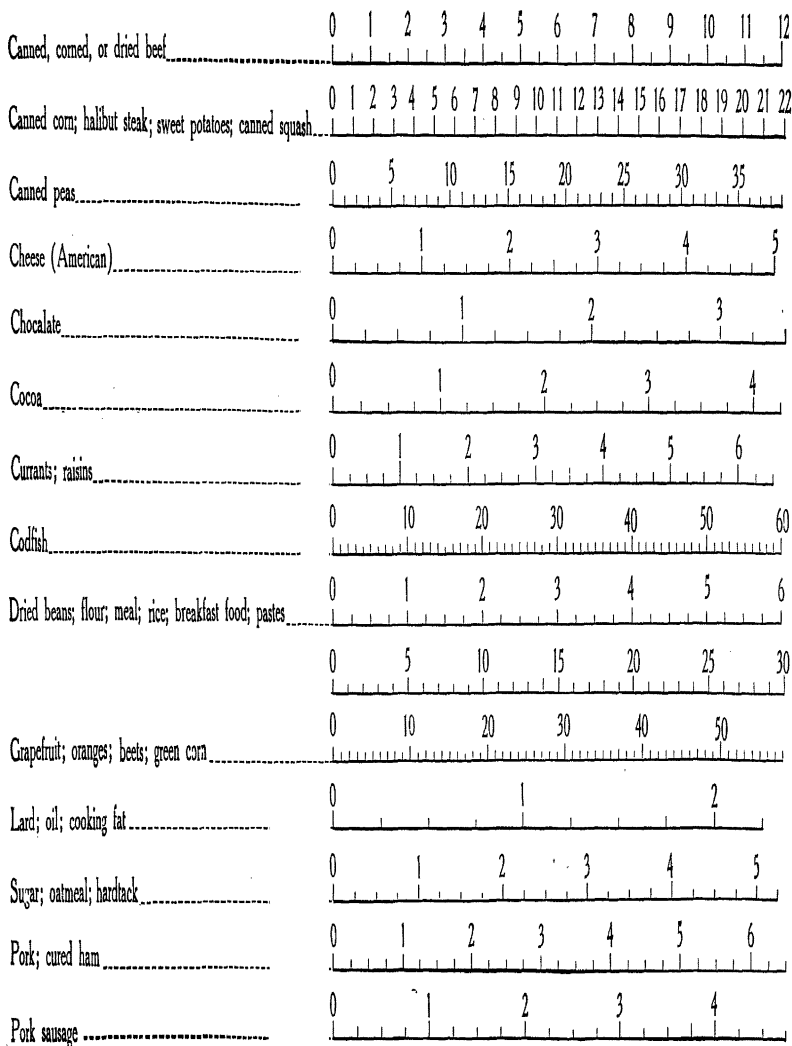


FIG. 17. Scale of calories of food-stuffs. Journal of the American Medical Association.



FOOD CALORIES

Scale for Rapid Determination of Calories

FIG. 17. — Continued.

He showed that the average surface area in square meters of infants, children, and adolescents is as follows:

Age, years	0	2	4	6	8	10	12	14	16	18	20
Square meters	0.15	0.53	0.64	0.71	0.77	0.86	0.99	1.15	1.35	1.54	1.73

Body activity affects metabolism (fig. 15), and food should be supplied proportionately (figs. 16, 17).

Boyd and Scammon: *The Growth of the Surface Area of the Human Body*, U. of Minn. Press (1934).

## DIVISION 4

### INTERNAL SECRETIONS AND METABOLISM

Internal secretions are produced by certain glands and poured into the blood. They have been called hormones.

The term "hormone" was first applied by Bayliss and Starling to:

**Secretin**, a hypothetical substance causing the pancreas to secrete and supposed to be a polypeptide. There is less evidence for the existence of **gastrin** causing gastric secretion.

Bayliss and Starling: *J. Physiol.* 28:61 (1903).

**Testicular Hormone** (androkinin,  $C_{18}H_{28}O_2$  or  $C_{19}H_{30}O_2$ , Bute-nant). Certain internal secretions are associated with sex. It is difficult to distinguish the sex of an early human embryo. The development of sexual differences appears to be associated with internal secretions since the differences tend to disappear or fail to develop on removal of the organ producing the internal secretions as by castration or spaying. Brown-Sequard was the first to attempt to make an active extract of the male glands of internal secretion.

Moore: *Am. J. Physiol.* 72:1 (1926); 87:436 (1928).

**Theelin** (see terpenes). A woman has a lower basal metabolism per square meter than a man owing to differences in internal secretions. The curve for female metabolism per square meter is below that of the male. Associated with the lower metabolism are the fattening tendency of the woman (especially the presence of subcutaneous fat) and a lower skin temperature. This lower skin temperature is very noticeable. A woman may stand in cold water much longer than a man without shivering. Heat loss

is inhibited by the lower skin temperature and subcutaneous fat, and shivering, which is a mechanism to produce more heat, is not called into play. The sex difference in production and loss of heat is evidently due to internal secretions of the sex organs.

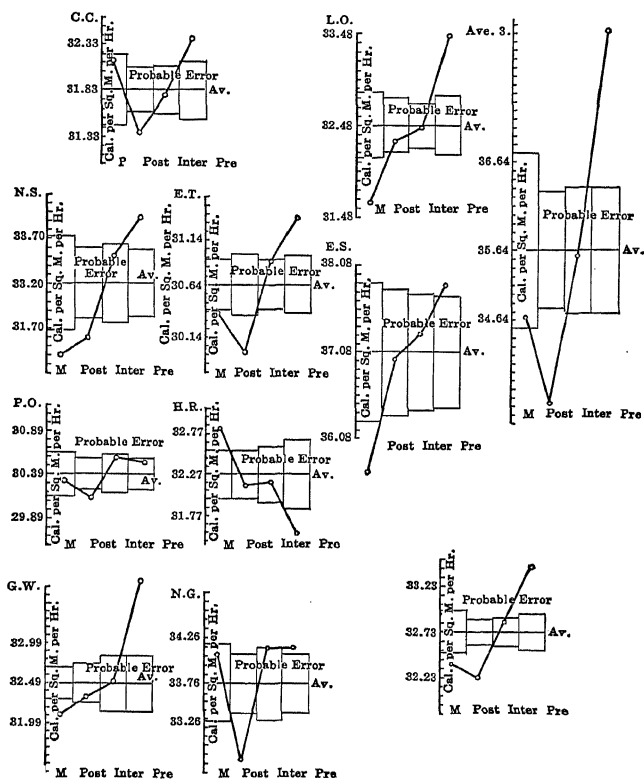


FIG. 18. Graphs of probable error of the basal metabolic rate in women throughout the menstrual cycle. Archives of Internal Medicine.

Allen and Doisy studied an active *ovarian hormone* (theelin) which causes estrus and changes in the uterus and is said to raise basal metabolism in castrated females and may explain menstrual variations in metabolism (fig. 18). The low B.M.R. is said to be due to low thyroid activity, but this is not quantitatively proved.

Allen and Doisy: *Physiol. Rev.* 7:600 (1927).

Frank: *Female Sex Hormone*, Thomas, Springfield, Ill. (1929).

**Corpus Luteum Hormones.** One causing pseudopregnancy (corporin) and one relaxing pelvic ligaments (relaxin) have been described.

Hisaw and Leonard: Am. J. Physiol. 92:574 (1930).

**Pituitrin**, an active hormone from the posterior lobe of the hypophysis or pituitary body at the base of the brain is used to hasten contraction of the uterus in order to reduce postpartum hemorrhage. It causes increase in blood pressure and during lactation a transitory increase in the flow of milk. It has been fractionated into *pitocin* which contracts the uterus and *pitressin* which affects blood pressure, and decreases the secretion of urine but increases the chlorides.

Bugbee and Kamm: Endocrinology 12:671 (1928).

**Growth Hormone of Anterior Hypophysis.** The age of puberty (age at which genital maturity takes place) has great influence on the growth of the skeleton. Late maturity leads to an overgrowth (especially the lower extremities are long), and premature maturity leads to undergrowth (with short lower extremities). This is due to the fact that the epiphyses in the first case are separated from the diaphysis by the growth zone, whereas in the other case they are united to the diaphysis too early. As soon as genital maturity occurs, the epiphyses unite and the growth is completed.

Growth is much more influenced by castration if it occurs before puberty. In such cases the growth does not cease at the physiological period, but the epiphyseal junctions remain open long after this time and the individual shows a disproportional overgrowth. The long bones of the extremities are most affected, for which reason the individual develops long arms and legs. The skull also shows changes with the result that the capacity of it develops less completely than is normal. This overgrowth is said to be due to removal of restraint of the gonad on the production of the anterior hypophysis growth hormone.

Benedict and Holmes showed that the removal of the anterior lobe of the pituitary body caused no evident change for some weeks, but after four weeks the metabolic rate gradually fell until a basal was reached 10 to 35% below normal. The animals took on fat tissue and seemed more sluggish.

Partial extirpation of the anterior lobe of this gland produces

in animals a condition of infantilism, increase in fat production, a decreased oxygen consumption, no further bodily growth, abortion of the pregnant uterus, body temperature about one degree sub-normal, a decreased reaction upon addition of adrenaline, and an increase in the eosinophiles of the blood. In man, in pathological conditions where the anterior lobe hypertrophies, a state of acromegaly (fig. 19) wherein the patient has disproportionately large feet and hands and an elongated and misshapen head develops.

Evans, Simpson, and Cornish:  
Proc Soc. Exp. Biol. Med. 27:101  
(1929).

**Uterus-stimulating hormone of anterior hypophysis** (prolan) has been studied especially in relation to the Zondek-Asheim test for pregnancy.

Zondek and Asheim: Endokrinologie 1:10 (1928).

Zondek: Zentr. Gynäkol. 53:834  
(1929).

**Pineal body** is supposed to be a gland of internal secretion (sex inhibitor), but Renton and Rusbridge observed no late effects of pinealectomy.

Horrax: Arch. Internal Med. 17:607 (1916).

Renton and Rusbridge: Proc. Soc. Exp. Biol. Med. 30:766 (1933).

**Adrenaline** from the *adrenal medulla* causes contraction of the arterioles, thus raising blood pressure, and is described under nitrogeneous bases.

Aub, Forman, and Bright in their work with adrenalectomy in cats showed that the removal of both adrenals caused a lowering of the total metabolism up to 25% in 48 hours. Control animals that were completely fasting showed a drop in metabolic rate of less than half the magnitude of that seen after adrenalectomy.

Removal of one adrenal caused a temporary drop in metabolism

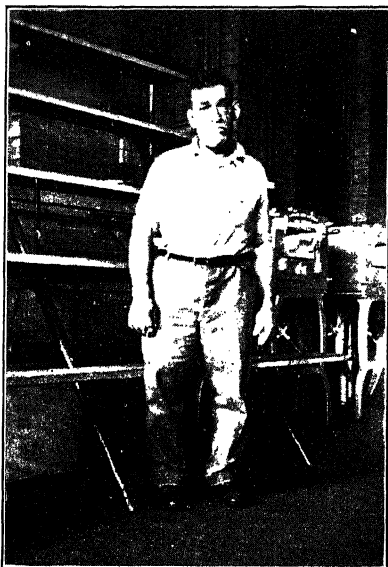


FIG. 19. Acromegaly.

but a gradual return to normal; denervation of the remaining adrenal showed a slow fall in the metabolic rate.

**Cortin.** Adrenal cortex contains a hormone whose absence causes Addison's disease.

Swingle and Eisenman: *Am. J. Physiol.* 79:679 (1927).

**Thymus** (thymocresin) is considered to be active until about the sixteenth year, after which it atrophies, but cases have occurred where it has been found in a functional condition as late as 60 years or longer after birth. A retardation of the atrophy of the thymus takes place after castration, which seems to show that the hormones of the mature gonads are directly or indirectly responsible for the disappearing of the thymus at puberty. A large thyroid always coincides with a small thymus, and vice versa.

Uhlenhuth: *J. Gen. Physiol.* 1:33 (1918).

**Thyroxine** (described under amino acids) has a marked effect on basal metabolism. It was found by A. V. Hill and Wood that certain cattle fattened on the same amount of food that others would grow thin on. What is the difference in these two cases? With thermocouples they measured the temperature of the skin and found that the skin temperature of those cows that had a fattening tendency was lower than that of the other cows. That means that the metabolism of the fattening cows was lower than that of the others. This may have been due to a difference in internal secretions since differences of basal metabolism from the standard are usually attributed to hormones. If the basal metabolism is not 36 to 40 Cal. per hour per square meter of body surface and there is no fever or prolonged starvation, the glands of internal secretion are examined. Since in myxedema and cretinism the basal metabolism is low and the skin cold, it is probable that the cows that fatten easily are deficient in thyroid.

Means estimated that myxedematous patients had an average basal metabolic rate of 60 to 80% of normal. Boothby and Sandiford found that in 41 patients with myxedema, 23 had a basal metabolic rate below 80% and 18 had a rate between 80 and 90% of normal. Means and Burgess found in 20 patients with myxedema and 4 with cretinism the average metabolic rate was 76% of normal. Boothby and Sandiford found that 76% of cases with colloid goiter had metabolism within normal range (not over 10% below normal).

In all these cases a rise in the basal metabolism was observed with the administration of thyroid. Möller reported that the rise takes place gradually and slowly, also that a few days elapsed before the rise was detectable. Plummer and Boothby found a rise in the basal metabolism after an injection of thyroxine. The rise was at its maximum a week after administration and dropped to the starting point in the course of about 50 days. They administered 16 mg. of thyroxine to a myxedematous patient with a metabolism of 50%. The metabolism rose to the maximum and fell gradually to the original level in 6 weeks. The 16 mg. of thyroxine caused a production of 16,125 Cal. above the quantity this patient would otherwise have produced. This is the equivalent of heat liberated in oxidation of 4.25 kg. of glucose. Subcutaneous injection of synthetic thyroxine causes a rise in metabolism in 2 hours which reaches a maximum in about 50 hours, and 1 mg. raises the basal metabolism 3%. The value of thyroid varies, as does also its destruction in digestion. In some cases, 5 grains of desiccated thyroid by mouth is equivalent to 1 mg. thyroxine subcutaneously.

Jordan conducted several tests on patients showing a basal metabolic rate above normal. He found 533 cases having a basal metabolic rate of 150 to 170%. Most of these were operated on; one-third of the cases dropped to a basal metabolic rate of 120% in 5 days after the completion of the operations and the rest dropped to normal inside of 6 months. In 320 having a basal metabolic rate of less than 150%, 6 months after operation practically all showed normal basal metabolic rates. (This subject is discussed more fully under Iodide.)

Boothby and Sandiford found the basal metabolism above 120% in practically all of 2,889 cases of Graves' disease. Means and Aub found that in 54 cases of Graves' disease the basal metabolic rate was between 120 and 190%. Möller, in 19 cases out of 89 classed as Graves' disease, found no rise in basal metabolism. The administration of thyroid lowers the basal metabolic rate in Graves' disease.

Boothby and Baldes: *J. Pharmacol. Proceedings* 25:139 (1925); *J. Biol. Chem.*, 54:47 (1922).

Marine: *Physiol. Rev.* 2:521 (1922).

**Parathormone.** The parathyroids produce an internal secretion that raises the blood calcium level. Removal of parathyroids

causes tetany due to low blood calcium. Death ensues unless parathormone is given or milk or other source of calcium is administered.

Collip: J. Biol. Chem., 63:395; 64:485 (1925).

**Insulin** (see glucose and polypeptides). The pancreas produces, besides the digestive secretions, an internal secretion, *insulin*, from the islands of Langerhans, which enables the body to burn glucose and store glycogen. It lowers the blood sugar (fig. 20) and is described under *d*-glucose and again under the polypeptides.

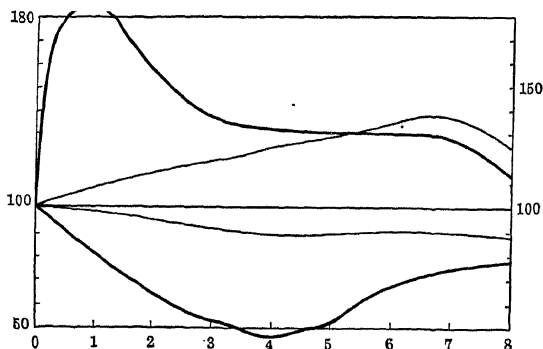


FIG. 20. Blood volume light curves and blood sugar changes heavy curves after taking insulin or glucose, based on the original values as 100. American Journal of Physiology.

## DIVISION 5

### IONIC EQUILIBRIA

**Hydrogen Ions.** Water dissociates into hydrogen ions and hydroxyl ions. The concentration of  $H^+$  can be determined in various ways and the  $OH^-$  calculated since  $\frac{[H^+] \times [OH^-]}{(HOH)} = K$  or  $[H^+] \times [OH^-] = K[HOH] = K_w = 10^{-14}$  at  $23^\circ$ . Acids dissociate into  $H^+$  and  $A^-$ .

$$\frac{[H^+] \times [A^-]}{[HA]} = K_a$$

The concentration of  $H^+$  may be determined by means of indicators, which are weak acids, by means of this equation.

A modified form of this equation is more useful in calculating the hydrogen ions. Let  $\alpha$  equal the degree of dissociation of the

acid. Then the above equation, assuming the acid to be in unit concentration, will be

$$\frac{\alpha[\text{H}^+]}{1 - \alpha} = K$$

At 50% dissociation

$$\log \frac{1}{[\text{H}^+]} = \log \frac{1}{K}$$

In general

$$\log \frac{1}{[\text{H}^+]} = \log \frac{1}{K} + \log \frac{\alpha}{1 - \alpha}$$

$\log 1/[\text{H}^+]$  is the log of the reciprocal of the hydrogen-ion concentration and is very easily determined by means of indicators and other means. Therefore Sørensen used it in much of his work and called it *pH*. The term *pH* is now so widely employed in medical literature that it cannot be ignored. Therefore, one must become familiar with this mathematical quantity and remember especially that when the hydrogen ions decrease in concentration the *pH* increases. Thus the *pH* of about 0.1*N* hydrochloric acid is 1; the *pH* of a neutral solution of absolutely pure, gas-free water at 23° is 7; and the *pH* of 0.1*N* carbon-dioxide-free sodium hydroxide is about 13.

By the use of an indicator, which is a weak acid with the ions colored and the undissociated molecules colorless, the depth of color signifies the amount of dissociation; hence  $\alpha$  may be determined by means of a colorimeter, and the *pH* calculated. Since the  $pH = \log \frac{1}{K} + \log \frac{\alpha}{1 - \alpha}$ , the graph of *pH* and  $\alpha$  will be the same type of curve for every indicator but the position of the curve will be determined by  $\log (1/K)$ .

If, instead of plotting  $\alpha$  against *pH*, we plot  $\log \frac{\alpha}{1 - \alpha}$ , we obtain a straight line, the slope of which is the same for all indicators, but the position is determined by  $\log (1/K)$ . The chart of this for various indicators is shown in fig. 21, which gives the value of  $\alpha$  so that one can determine the *pH* with the colorimeter with any indicator he may choose, provided that the undissociated molecules of the indicator are colorless. Since the indicators are not in normal solution an error arises, but this is usually not very significant.



DISSOCIATION CONSTANTS AND SALT ERRORS OF  
MONOCHROMATIC INDICATORS

Indicator	log (1/K)	Salt Concentration			
		low	0.05 M	0.15 M	0.5 M 0.6 M
Picric acid.....	0.7				
(changes to orange in 2 N NaOH and fades)					
Quinaldine red.....	2.6				
4, 6 Dinitroguaiacol.....	3.4				
2, 6 Dinitrophenol.....	3.69				
Pinocyanol.....	3.7				
2, 4 Dinitrophenol.....	4.06-4.08	3.95	3.98	3.88	
Red-violet, 5 RS.....	4.08		4.76		
2, 3 Dinitrophenol.....	4.87		4.76	4.71	
2, 5 Dinitrophenol.....	5.16	5.15	5.08	5.01	
3, 4 Dinitrophenol.....	5.35		5.30	5.25	
Orthochrome T.....	6.7				
Orthonitrophenol.....	6.0				
Dinitrobenzoyleneurea.....	7.0				
Paranitrophenol.....	7.80-7.22	7.22	7.17	7.03	8.2
Metanitrophenol.....	8.35	8.35	8.24	8.19	9.8
Orthocresoltetrachlorophthalein.....	8.75				
Cresolphthalein.....	9.4				
Phenolphthalein.....	9.7-9.76		9.6	9.5	
Nitramine (fades in alkali).....	12.4				
1, 3, 5 Trinitrobenzene.....	12.8				
Tetrabromophenolphthalein.....	8.5				
<i>o</i> -Cresol tetrachlorophthalein.....	8.75				
<i>o</i> -Cresolphthalein.....	9.0				
1, 2, 3 Xylenolphthalein.....	9.0				
Thymolphthalein.....	9.9-fades				
SC <sub>3</sub> H <sub>3</sub> phenolphthalein.....	9.2				
SC <sub>4</sub> H <sub>3</sub> phenolphthalein.....	9.2				
SC <sub>6</sub> H <sub>3</sub> phenolphthalein.....	9.6				
Dibromothymoltetrachlorophthalein.....	8.6				

There are great discrepancies in the above table in data from different authors.

In order to determine log (1/K) for an indicator under the experimental conditions (temperature and salt content) the electrode method is used.

It has been shown by physical chemists that the activity of hydrogen ions as affecting different properties of a solution may have different coefficients, and hence we will define activity as that affecting electrode potentials and indicators and particularly distinguished from conductance ratios. The simplest method of

determining hydrogen-ion activity is by means of a pair of hydrogen electrodes (fig. 22) connected through a salt bridge on one hand and on the other hand through a potentiometer. If the two solutions have the same hydrogen-ion activity and temperature they will be at the same potential, and hence the hydrogen-ion activity of an unknown solution may be determined by finding a solution of known hydrogen-ion activity in the other electrode,

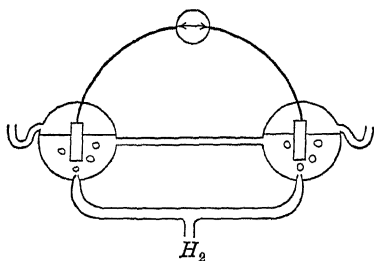


FIG. 22. Hydrogen electrodes. American Naturalist.

which will be at the same potential. On the other hand, if one electrode contains ten times as much hydrogen-ion activity as the other, there will be a difference of 59 millivolts in the potential of the two electrodes. Thus each unit change in  $pH$  causes a change in potential of 59 millivolts.

Sørensen, however, standardized the solution in his reference electrode by means of conductance ratios, and hence all his values of  $pH$  must be corrected by adding 0.04.

The practical use of the hydrogen electrode has met with some difficulties. In the first place, the surface of the electrode must be one which will absorb a store of hydrogen in the monomolecular form and quickly reach saturation. In order to prevent the loss of hydrogen through its penetration into the interior of the electrode, electrodes have been made of gold, which absorbs very little hydrogen, and then electroplated with a metal which absorbs hydrogen more rapidly. Palladium may be used for plating, but it is attacked by certain solutions. It may be removed with nitric acid and deposited as at first. A platinum surface has to be laid down under certain conditions which are empirically determined, but it may become poisoned so that it reaches equilibrium slowly in oxidizing solutions, and it cannot be removed with nitric acid. About the only way to treat such an electrode is to replating it on top of the old poisoned surface. Iridium seems to be an excellent metal for the surface of the electrode.

Whether the surface is applied smooth and bright or rough and black affects the determinations in various ways, particularly in

the presence of oxidizing solutions. The electrode surface acts as a catalyst, causing a union of hydrogen with the oxidizing substance, which thus prevents a saturation of the electrode with hydrogen. If this catalytic action is very rapid, however, and hydrogen is supplied in gaseous form, it should be possible to reduce the whole solution by means of the electrode until saturation with hydrogen is possible.

A particular difficulty in biochemical measurements arises from the fact that the passage of carbon dioxide into the hydrogen may change the hydrogen-ion concentration in the solution. In the Clark electrode the hydrogen may be retained while part of the solution is changed, in this way bringing new samples of the solution in relation to the hydrogen which has come to equilibrium with the earlier samples. If the solution is viscous, as blood plasma, the McClendon electrode vessel may be used for shaking the hydrogen with some of the solution in one compartment, then with a new portion of the solution in the second compartment containing the electrode. For solutions which are not viscous the electrode must be completely immersed when taking the reading. If the solution contains oxygen the electrode must be shaken until all the oxygen is reduced by means of the hydrogen. It is therefore unwise to use rubber stoppers in electrode vessels or rubber connections for hydrogen gas since both oxygen and carbon dioxide may diffuse through the rubber. The hydrogen from a cylinder may be passed over heated platinized asbestos to remove the oxygen.

If the two hydrogen electrodes, one containing a standard solution of hydrogen ions, are at different hydrogen-ion activities, there will be a diffusion potential in the salt bridge connecting the electrodes. This potential may be reduced by making the salt bridge of a saturated solution of potassium chloride, since the potassium ions travel at about the same rate as the chlorine ions, and most of the current is carried by potassium and chlorine ions. If, however, the hydrogen-ion activity in one of the electrodes has a very high value, an appreciable amount of the current will be carried by hydrogen ions which will give rise to a higher diffusion potential. The same would be true of hydroxyl ions in very alkaline solutions. In most biochemical work, however, the solutions are often near neutrality, and hence a salt bridge of saturated potassium chloride may reduce the diffusion potential to a low

value. Diffusion potentials not only cause error in the determination but also they may vary from moment to moment and give an inconstant reading. This is due to a change in the character of the junction brought about by diffusion.

In order to set up junctions that are constant, various forms of flowing junctions have been devised in which the current opposes the effect of diffusion on the distribution of ions. Although the liquid-junction potential may be reduced by using a saturated potassium chloride solution, it is not absolutely eliminated, and furthermore, it is not constant but changes with time. Therefore, it seems a waste of time to attempt to get hydrogen electrode readings in smaller units of potential than a millivolt.

In order to maintain constancy of the standard electrode, it should be filled with a buffer solution, which is essentially a mixture of an equivalent number of molecules of a weak acid and its salt with a strong base.

A calomel electrode may be substituted, which is essentially pure mercury covered with a layer of pure calomel in a potassium chloride solution. The concentration of the potassium chloride affects the solubility of the calomel and hence the potential of the electrode. In the 0.1N KCl calomel electrode the electric conductivity of the potassium chloride solution is so low as to reduce the accuracy of the readings, hence the normal potassium chloride calomel electrode or the saturated potassium chloride calomel electrode have been substituted. In the last named the calomel is mixed with crystals of potassium chloride. Since the solubility of calomel is affected by temperature, and time is required for equilibrium, there is a time-lag in all calomel electrodes after a change of temperature. This lag is greatest in the saturated potassium chloride calomel electrode, since the temperature affects the solubility of potassium chloride as well as calomel.

This is just another reason for working at constant temperature. Whether a calomel electrode or a standard hydrogen electrode is used at constant temperature, the  $pH$  is a linear function of the potential difference, and addition is all that is required in constructing a table for converting millivolts into  $pH$ .

In routine determinations, if it is necessary to have flowing junctions or other elaborate arrangements, the time required for each determination may be very great. For this reason other methods have been used for determining hydrogen-ion activities,

and the hydrogen electrode has been reserved for standardization.

Of the other electrode methods, the quinhydrone electrode is perhaps the most reliable. The quinhydrone electrode consists of a platinum wire dipping into an unknown solution, which is saturated with quinhydrone. This is connected by a salt bridge to a calomel electrode. Often the platinum wire is moistened and dipped into the quinhydrone and stirred in the unknown solution. Sometimes the quinhydrone tends to float on top as a dry powder and is difficult to wet. The liquid-junction potential vitiates this method as it does the hydrogen electrode method.

One is here measuring an oxidation-reduction potential which is influenced by the hydrogen-ion concentration. Quinhydrone is a combination of one molecule of quinone and one of hydroquinone and on dissociation gives rise to the same concentration of quinone and hydroquinone, and to this extent the oxidation-reduction potential is constant and the hydrogen-ion activity the only variable. If, however, the solution contains substances which will oxidize hydroquinone or reduce quinone appreciably before the potential readings can be made, the method cannot be used. The sources of error in the method are the liquid junction, impurity of the quinhydrone, failure to saturate the solution with quinhydrone, loss of carbon dioxide, and failure to regulate the temperature.

The quinhydrone electrode was standardized by means of the hydrogen electrode, and it seems probable that each laboratory should standardize it independently. Conversion tables can be made in the same way as for the hydrogen electrode. After standardizing at two hydrogen-ion activities (since the  $pH$  is a linear function of the potential), it is not even necessary to know the formula.

Escape of carbon dioxide, will change the hydrogen-ion activity in the quinhydrone electrode in the same way as it will in the hydrogen electrode, but since it is not necessary to have a gas phase, this escape may be easily prevented if some arrangement for stirring-in the quinhydrone without opening the apparatus is provided. Rubber stoppers and connections would be undesirable here also because of their permeability to carbon dioxide, but as a rule the reading can be taken very quickly, and perhaps the best approach to accuracy is made by working rapidly.

It was shown by McClendon and Magoon that the hydrogen

ions in bicarbonate solutions or blood plasma do not change appreciably with change in temperature between 20° and 40°. But the hydroxyl ions are greatly influenced by temperature. Furthermore, the colors of indicators in the blood plasma (or in any buffer solution) are influenced by temperature.

Neutral salts affect the dissociation of indicators without change of *pH* owing to the effect of their ionic strength on the activity coefficients of the indicator, and probably do not change the dissociation constant. But the salt effect may be expressed in terms of change in apparent dissociation constant.

Protein affects the color of the indicator. The effect of protein is minimized by dilution, but dilution also changes the hydrogen ions. Since the indicator method must be standardized by the hydrogen electrode, the standardization eliminates the error of dilution when using the undiluted solutions with the hydrogen electrode and the diluted with the indicator. Blood plasma should be diluted between 12 and 20 times with carbon-dioxide-free water. The dilution must be the same in the standardization as in the other determinations.

Clark: *The Determination of Hydrogen Ions*, third edition, Williams & Williams, Baltimore (1928).

McClendon, *Am. Naturalist* 64:289 (1930).

The hydrogen-ion concentration of the blood has been studied considerably. L. J. Henderson showed that the blood acts as a bicarbonate solution — in other words

$$\frac{[\text{H}^+] \times [\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} = K$$

This is called Henderson's equation. If we assume that the bicarbonate is 100% dissociated, the total concentration of bicarbonate =  $\text{HCO}_3^-$ . Hasselbalch therefore modified this equation to

$$\frac{[\text{H}^+] \times [\text{bicarbonate}]}{[\text{H}_2\text{CO}_3]} = K_1$$

which is called the Henderson-Hasselbalch equation. It is impossible to determine  $\text{H}_2\text{CO}_3$  accurately. According to Henry's law,  $\text{H}_2\text{CO}_3$  is proportional to  $\text{CO}_2$ -pressure, and we can write:

$$\frac{[\text{H}^+] \times [\text{bicarbonate}]}{\text{CO}_2\text{-pressure}} = K_2$$

From this equation we can determine the hydrogen ions by determining the amount of bicarbonate of the blood and partial pressure of  $\text{CO}_2$ . This method would be much more difficult than the indicator method that we use in the laboratory, but the equation serves to give us the picture of what happens during the circulation of the blood, assuming the bicarbonate to be constant (changing immeasurably slowly by kidney excretion), but the  $\text{CO}_2$ -pressure changes rapidly by excretion in the lungs and formation in the tissues. The  $\text{CO}_2$ -pressure in the lungs is about 45 mm. Hg or about 6% of an atmosphere. The  $\text{CO}_2$ -pressure in the tissues is higher than that, owing to the production of  $\text{CO}_2$ . When the blood reaches the lungs it is spread out over 125 sq. m. of surface, and the  $\text{CO}_2$ -pressure equals that in the lungs. If the  $\text{CO}_2$ -pressure is reduced in the blood, the hydrogen-ion concentration is reduced.

Most people are more familiar with pH values than with  $\text{H}^+$  concentration. The above equation may be written

$$\text{pH} = \log \frac{1}{K_2} + \log \text{bicarbonate} - \log \text{CO}_2\text{-pressure}$$

$$\text{at } 37^\circ: \quad 7.47 = 7.64 \quad + \quad 1.48 \quad - \quad 1.65$$

In the blood the  $\text{pH}$  = about 7.47. Bicarbonate = about 30 millimols per liter, and  $\log$  bicarbonate is then 1.48 and  $\text{CO}_2$ -pressure about 44.7 mm. Hg; hence  $\log \text{CO}_2\text{-pressure} = 1.65$ .  $\log (1/K_2) = 7.64$  at  $37^\circ$ .

The variation of arterial and venous blood is rather small. The question arises how these conditions in the blood are kept so constant. The bicarbonate concentration in the blood is kept within constant limits in health by the ability of the kidney to excrete the same amount of fixed acid and strong base that are absorbed from the gut.

The hydrogen ions are kept within fairly constant limits by the fact that the respiratory center in the medulla of the brain acts as a regulator of hydrogen-ion concentration in the blood. This was first shown by Winterstein, who caused newborn pups to start breathing immediately by injecting  $\text{HCl}$  into their veins. When hydrogen ions increase, the respiratory center is stimulated and we breathe faster, blowing more  $\text{CO}_2$  (equivalent to carbonic acid) out of the lungs. This reduced the pressure of  $\text{CO}_2$  in the blood and therefore reduces the hydrogen ions. On the other

hand, if the pressure of  $\text{CO}_2$  is suddenly reduced in the blood, the hydrogen ions are decreased and the desire to breathe disappears. One can illustrate this by attempting to make a fire burn by blowing violently on it. After several minutes of violent blowing one has no desire to breathe for an appreciable length of time.

The other point is illustrated by breathing in and out of a large paper bag. The  $\text{CO}_2$  is breathed in again, and consequently, when the  $\text{CO}_2$ -pressure in the blood increases, the hydrogen ions increase, and the desire to breathe increases until violent panting results.

With a change in hydrogen-ion concentration in the blood plasma there is a change in hydrogen-ion concentration in the blood corpuscles — perhaps in all cells of the body because the product of  $[\text{H}^+] \times [\text{HCO}_3^-]$  in the plasma and the corpuscles is equal, since  $\text{CO}_2$  can freely diffuse into and out of the corpuscles. This may be expressed by the equation

$$[\text{H}^+] \times [\text{HCO}_3^-] = K [\text{H}_2\text{CO}_3]$$

Henderson: *Blood, A Study in General Physiology*, Yale Univ. Press, New Haven (1928).

**Chloride Shift.** Although the blood corpuscles are impermeable to  $\text{Na}^+$ , they are not impermeable to  $\text{Cl}^-$ . In 1867, Zunz showed that, if the blood is saturated with  $\text{CO}_2$  and then centrifuged, *the bicarbonate of the blood plasma is increased*. This has been shown to be due to the passage of chloride ions into the cells and the passage out of bicarbonate anions, thus changing the sodium of the plasma to  $\text{NaCl}$ , this phenomenon being known as the *chloride shift*.

This can be explained on the basis of Donnan's theory of membrane equilibrium in a qualitative way. Little can be done in a quantitative way because we may not know all the quantities accurately enough and the case is very complicated by reason of the fact that there are non-diffusible ions on *both* sides of the membrane, the principal ones being  $\text{Na}^+$  in the plasma and  $\text{K}^+$  in the corpuscles. The 40% of hemoglobin acts as a non-diffusible anion and greatly overbalances the 8% of protein of the plasma.

The chloride shift may be pictured as follows: If  $\text{CO}_2$  gas is bubbled through blood it passes not only into the plasma but also into the corpuscles, and under these conditions the bicarbonate concentration in the corpuscles is greater than that in the plasma

and the bicarbonate anions pass from the corpuscles to the plasma. In order to satisfy electrical neutrality, the same number of chloride ions pass from the plasma to the corpuscles. When  $\text{CO}_2$  is removed from the blood the bicarbonate concentration in the corpuscles decreases owing to the fact that  $\text{K}^+$  combines with  $\text{Hb}^-$  to form potassium hemoglobinate instead of potassium carbonate.

When the blood passes through the capillaries in the tissues  $\text{CO}_2$  enters the blood and passes into the corpuscles and potassium hemoglobinate is changed into potassium carbonate.  $\text{HCO}_3^-$  passes into the plasma and  $\text{Cl}^-$  passes into the corpuscles to satisfy electric neutrality. In its passage through the lungs  $\text{CO}_2$  is blown out of the blood. Then the chlorine ions diffuse out of the corpuscles. Thus the bicarbonate of the plasma is partly replaced by chloride.

The distribution of ions in arterial blood is as follows:

$$\begin{aligned}\text{In the corpuscles } [\text{H}^+] &= 4.9 \times 10^{-8} (p\text{H} = 7.31), \\ [\text{HCO}_3^-] &= 0.018 N, \\ [\text{H}^+] \times [\text{HCO}_3^-] &= 0.09 \times 10^{-8}.\end{aligned}$$

$$\begin{aligned}\text{In the plasma } [\text{H}^+] &= 3.6 \times 10^{-8} (p\text{H} = 7.45), \\ [\text{HCO}_3^-] &= 0.025 N, \\ [\text{H}^+] \times [\text{HCO}_3^-] &= 0.09 \times 10^{-8}.\end{aligned}$$

ARTERIAL BLOOD	
<i>Corpuscles</i>	<i>Plasma</i>
$[\text{H}_2\text{CO}_3] = a$	$[\text{H}_2\text{CO}_3] = a$
$[\text{H}^+] = 4.9 \times 10^{-8} (p\text{H} = 7.31)$	$[\text{H}^+] = 3.6 \times 10^{-8} (p\text{H} = 7.45)$
$[\text{HCO}_3^-] = 0.018 N$	$[\text{HCO}_3^-] = 0.025 N$
Product = $0.09 \times 10^{-8}$	Product = $0.09 \times 10^{-8}$

What is the force which causes the concentration of the hydrogen ions to be different on the two sides of the membrane whereas  $\text{H}^+$  is free to diffuse? This is a complicated case as we mentioned above, but by simplification it may be pictured as due to the indiffusibility of the hemoglobin which bears negative charges and attracts hydrogen ions which have positive charges. This may also explain the distribution of chloride ions. The chloride ions are more concentrated in the blood plasma where there are 370 mg. (100 milli-equivalents) per 100 cc. whereas in the corpuscles there are about 50 milli-equivalents of chloride ions. We may assume

that the negative charge on the hemoglobin repels the chloride ions and drives them into the plasma.

When the blood passes through the capillaries,  $\text{CO}_2$  passes into it and into the corpuscles. This causes several changes. In the first place, in the corpuscle  $[\text{H}^+]$  increases to  $5 \times 10^{-8}$  ( $\text{pH} = 7.3$ ) owing to diffusion of  $\text{CO}_2$ , and  $[\text{HCO}_3^-]$  increases to  $0.022 N$  owing to the fact that carbonic acid decomposes  $\text{KHb}$  and forms  $\text{K}^+$  and  $\text{HCO}_3^-$ .

$$[\text{H}^+] \times [\text{HCO}_3^-] = 0.11 \times 10^{-8}$$

$[\text{HCO}_3^-]$  would be still higher in the corpuscles if it had not partly diffused into the plasma where it increases to  $0.028 N$ . Thus  $\text{HCO}_3^-$  diffuses against a concentration gradient, being repelled by the electric charge on the hemoglobin. The product  $[\text{H}^+] \times [\text{HCO}_3^-] = 0.11 \times 10^{-8}$  on both sides of the membrane. In the plasma  $[\text{H}^+] = 3.8 \times 10^{-8}$  ( $\text{pH} = 7.42$ ).

Corpuscles	Membrane	Plasma
$[\text{H}^+] = 5 \times 10^{-8}$ ( $\text{pH} = 7.3$ )		$[\text{H}^+] = 3.8 \times 10^{-8}$ ( $\text{pH} = 7.42$ )
$[\text{HCO}_3^-] = 0.022 N$		$[\text{HCO}_3^-] = 0.028 N$
Product = $0.11 \times 10^{-8}$		Product = $0.11 \times 10^{-8}$

At the same time the corpuscles swell slightly. This is due to increased osmotic pressure caused by the fact that the sum total of all the osmotic substances is greater in the venous blood than in the arterial blood. This is probably mainly due to the increase of  $[\text{HCO}_3^-]$  and  $[\text{Cl}^-]$  in the corpuscles. If this chloride shift is to be explained by Donnan's hypothesis, then Donnan's ratio would be:

$$r = \frac{[\text{H}^+] \text{ serum}}{[\text{H}^+] \text{ corpuscle}} = \frac{[\text{Cl}^-]_c}{[\text{Cl}^-]_s} = \frac{[\text{HCO}_3^-]_c}{[\text{HCO}_3^-]_s} = \frac{[\text{OH}^-]_c}{[\text{OH}^-]_s}$$

Attempts have been made to verify this scheme, but the situation is more complicated than it at first seems. In the first place it is not the concentration in the older sense that is effective but the thermodynamic "activity" of the ions. The activity of the ions is dependent on the total ionic strength and perhaps other factors.

It seems probable that  $r =$  about 0.72 for arterial blood and 0.76 for venous blood, but the data on chloride, when interpreted with the old idea of concentration, would make  $r$  very much

smaller. No matter how many non-diffusible ions are present, Donnan's theory necessitates that  $r$  is the same for all diffusible ions.

Steggerda showed that muscles in 1% NaCl absorb  $\text{Cl}^-$ , which would be expected if we assume that the permeability of the muscles is similar to that of the blood corpuscles, especially that the muscles are permeable to  $\text{Cl}^-$ . In 1% NaCl,  $\text{Cl}^-$  is much more concentrated than in blood plasma although the osmotic pressure is about the same. We should expect that  $\text{Cl}^-$  would diffuse into the muscle in exchange for  $\text{HCO}_3^-$  diffusing out.

Cameron and Walton: Trans. Roy. Soc. Canada 22: Section V, p. 1 (1928).

Dautrebande, Lucien, and Davies: J. Physiol. 57:36 (1922).

Denis and Sisson: J. Biol. Chem. 46:483 (1921).

Hastings, Sendroy, McIntosh, and Van Slyke: J. Gen. Physiol. 8:70 (1927).

Van Slyke: Factors Affecting the Distribution of Electrolytes, Water, and Gases in the Animal Body, Lippincott, Philadelphia (1926).

**Hydrogen-Ion Concentration and Enzyme Action.** All living cells contain enzymes, and enzymes exist in the cell-free portion of body fluids—thus the saliva contains an enzyme or mixture of enzymes called *ptyalin*. It acts best at about *neutrality* or in a nearly neutral solution ( $p\text{H}$  7). The  $p\text{H}$  of the freshly secreted saliva is about 6.8 but increases when the mouth is opened and  $\text{CO}_2$  allowed to escape. From a practical standpoint, we may say that the saliva is not necessary in the digestion of carbohydrates because the *amylase* of the pancreatic juice is thoroughly capable of doing this work. Perhaps the function of the *ptyalin* is to digest the starch remaining between the teeth after a meal, producing glucose, which washes away. Otherwise bacteria would change the starch to lactic acid, which might dissolve the teeth. Uncooked starch digests slowly, particularly if it is inside cell walls. The enzyme cannot get in through the cellulose and the starch cannot get out. Some of the starch of string beans is digested, however. It seems possible that bacteria attack the cellulose and make holes in it before the digestion of starch commences.

When the food passes into the stomach, salivary digestion continues only until the acid of the gastric juice penetrates the food mass, because the pure gastric juice is 0.1  $N$   $\text{HCl}$  or  $p\text{H}$  of about 1. The decrease in  $p\text{H}$  of the gastric content shows a characteristic curve (fig. 23). The pepsin of the stomach is destroyed

in time by a  $pH$  of 1, and the destruction rapidly increases as  $pH$  is reduced to less than 1. Therefore, in studying the rate of peptic digestion two processes are involved: the destruction of the pepsin and the rate of action of the remaining pepsin. With both processes going on, pepsin acts best at a  $pH$  of about 1.5. As the  $pH$  is increased from 1.5, peptic activity decreases until somewhere between 4 and 5, the rate becomes so small it cannot be easily measured. Numerous examinations of gastric contents indicated that about 0.2% HCl was usually found whereas 0.1  $N$  HCl is 0.36% HCl. Evidently, some of it is neutralized. The

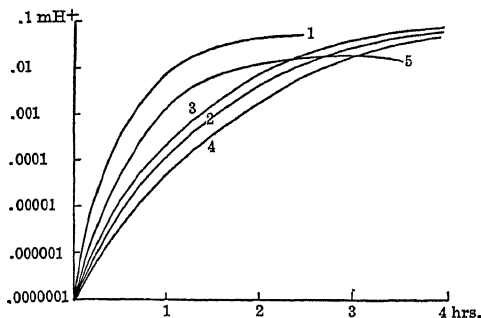


FIG. 23. Gastric hydrogen ions in 5 adults. American Journal of Physiology.

saliva contains bicarbonate, and several liters a day are secreted. The food also binds acid but even in the absence of food the acid becomes partly neutralized.

It appears as though high acidity is objectionable in the stomach. In persons who habitually lack acid in the stomach (achlorhydria), swallowing of 0.1  $N$  HCl produces great discomfort. The discomfort of the empty stomach, otherwise known as hunger, is thought by Carlson to be due to contractions. No one has shown that the high acidity produced discomfort in normal persons but this acidity does not remain as a rule. In the first place the empty stomach does not secrete acid at a rapid enough rate to make the neutralizing power of the swallowed saliva negligible and, secondly, as was shown by Boldyreff, under such conditions there may be a regurgitation of pancreatic juice, which contains bicarbonate and which neutralizes some of the acid.

High acidities are reached in the stomach during pyloric spasm.

Perhaps the spasm of the pylorus may be so intense as to prevent regurgitation. Perhaps the irritation causing the spasm may cause increased secretion of acid. We must, at any rate, distinguish between the acidity of the gastric juice and the acidity of the gastric contents. The acidity of the gastric juice in health is  $0.1N$  HCl;  $0.1N$  HCl in the gastric contents is called hyperacidity. Whether this acidity is irritating to the normal stomach, or whether some other irritation caused the spasm (which caused the hyperacidity) and is the primary factor, has not been determined.

The clinical problem is complicated by the habit of swallowing air. Ordinarily some air is taken in the stomach with food and drink and the dome of the stomach is always filled with air, but some persons habitually swallow air and complain of "hyperacidity." In this condition they may have a spasm of the cardia and may not be able to let the air out. They take a little sodium bicarbonate in water and the air rushes out. The physics and chemistry of this are not very well understood.

When a heavy meal is eaten the  $pH$  of the gastric juice changes from about 1 to about 6. Then the gastric content gradually decreases in  $pH$  as the food is held in the stomach. The final  $pH$  when the food leaves the stomach varies in individuals on both sides of an average of about  $pH$  2. The heavier the meal the longer the food stays in the stomach and the longer the time required for secretion of enough acid to bring the  $pH$  to about 2 (fig. 23). It thus appears as though hydrogen ions regulate the staying of food in the stomach.

This is not the only factor influencing the pylorus, however, because a solid body may mechanically cause the pylorus to close tightly, for a little while at any rate. This is shown in attempts at getting tubes down into the duodenum. In the infant's stomach the food leaves it before a very high acidity is reached but the gastric content of the pyloric end of the stomach is more acid than that of the fundus.

The enzymes of the stomach are chiefly concerned in digesting protein. The first action on the casein of milk is to clot it. In the infant and the calf the stomach seems to be an organ purely for the purpose of clotting the milk. The enzyme responsible for it is called rennin. The purest preparations of pepsin that have been studied in this way clot milk. They may contain some

rennin or we may suppose that milk may be clotted by two enzymes — rennin and pepsin. The old argument as to whether rennin and pepsin are the same has not been decided. If we call them the same we must assume that these preparations of the gastric enzyme behave somewhat differently. Whether this is due to difference in the enzyme itself or to impurities has not been decided. Northrop has crystallized pepsin.

If protein is put in acid it binds considerable of the acid. Such protein is called acid-meta-protein (acid-albumin) and it is more easily digested by pepsin when added to a neutral solution than is ordinary protein (albumin). Whether the acid has affected the protein substrate, or whether the dissociation of hydrogen ions from the acid-albumin affects the enzyme, has not been determined. Much labor has been expended in determining the combined HCl of the gastric contents but no accurate method for its determination has been devised and it is questionable whether it has any important clinical significance. The *pH* of the saliva and the final *pH* of the gastric contents seem to be the optimum for the enzymes concerned.

It appears as though there were enough hydrogen ions in the chyle passed into the duodenum for peptic digestion to go on for a while, even though there was not an appreciable amount of peptic digestion in the infant's stomach. The *pH* of the intestine increases from the stomach toward the ileocecal valve, and in animals with a long intestine may become neutral or alkaline before reaching this valve. In animals with a short intestine, however, the contents are practically always on the acid side of neutrality as long as food particles are present. This may be studied by passing a rubber tube many feet down into the intestine (fig. 24).

The empty intestine, however, may become alkaline, as shown in the Mayo Clinic. The small amount of fluid in the intestine not receiving food from the stomach is probably succus entericus, and it has long been known that this is alkaline in reaction as is also the pancreatic juice. The bile is more nearly neutral. The pancreatic juice and succus entericus contain bicarbonate, which neutralizes the HCl and amino acids, and acids due to carbohydrate fermentation. So the acidity is reduced as one goes toward the ileocecal valve and the content may become alkaline in the cow and rabbit, for instance, but never so in the dog as long as

there is gastric juice coming from the stomach or amino acids arising from the splitting of proteins.

The intestine becomes more acid with certain carbohydrates than with proteins. Bacteria may decarboxylate amino acids and produce amines or basic substances in the intestine but some deaminization occurs, producing fatty acids.

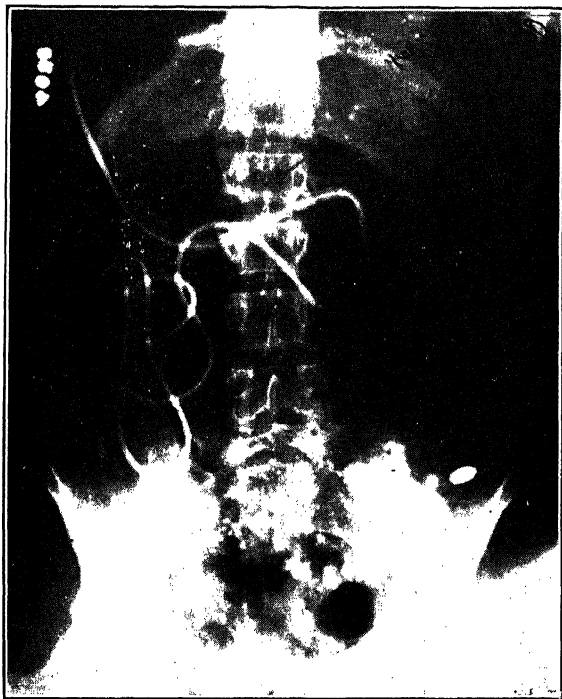


FIG. 24. Roentgenogram of 7 foot tube in jejunum. The oval in lower right is a 6 g. iron weight that was attached to the tube with 30 day catgut but has been digested off.

The fatty acids arising from the fermentation of carbohydrates are laxative and irritating to the gut. It has not been shown, however, that this is due to the hydrogen ions, in fact the ingestion of sodium acetate acts as a violent purge when in sufficient quantities.

The large intestine is not a place for digestion of food but

merely one for re-absorption of water. The pH of the intestinal contents cannot be the exact optimum for all processes because the optimum changes from enzyme to enzyme, and even for the same enzyme with different substrates. Trypsin acts best on casein on the acid side (pH 6) but on fibrin (pH 8) and gelatin (pH 9.7) on the alkaline side of neutrality. Amylopsin works best at nearly neutral reaction but steapsin is probably favored by alkali.

Michaelis: *Die Wasserstoffionenkonzentration*, second edition, Berlin (1924).

**pH and the Colloidal State of Food and Drugs.** Protein has an isoelectric point at which swelling is least. Swelling seems to favor digestion of the protein. If an electric current is passed through an ampholyte solution at the isoelectric point, ampholyte will not pass to either the anode or the cathode or else one-half will go to the anode and the other half to the cathode. The difference depends on the acid and basic dissociation, and three conditions are possible in an ampholyte, depending on the magnitude of  $K_a$  and  $K_b$ . In the first condition at the isoelectric point both anions and cations are present and go to both poles. The second possibility is that only neutral molecules exist at the isoelectric point and no migration takes place. The third condition is that only neutral molecules exist at the isoelectric point and on the two sides of the isoelectric point, called the isoelectric zone, and no migration takes place if the pH is anywhere in this zone.

On the two sides of the isoelectric zone or point, swelling of a protein increases. Thus glue can be made out of casein (which clots at the isoelectric point) by putting in either acid or base in a sufficient amount.

Agar-agar is a carbohydrate gel. If it is too acid it will liquefy, which is not entirely due to removal from the isoelectric point, but the agar is combined with calcium and there is competition of the agar and the added acid for this base. Agar robbed of the calcium liquefies. The same is true of pectin, which is the calcium salt of pectic acid and is the gel of fruit jellies. If calcium is removed by too much acid it will dissolve, but it will stand more acid than agar. Gels are used in medicine for holding or applying drugs as in suppositories. Many failures may be due to the fact that the gel liquefies because of heat or acidity.

Gortner: *Outlines of Biochemistry*, Wiley, New York (1929).

Pauli: *The colloid chemistry of proteins*, *Kolloid Z.* 31:252 (1922).

**pH of Urine.** Urine is a phosphate solution in so far as pH is concerned, i.e., the phosphates are its chief buffers. It is only in alkaline urine that bicarbonate is found. By adding alkali to phosphate until phenolphthalein turns red,  $\text{Na}_2\text{HPO}_4$  (pH 9) is obtained; and by adding acid until brom phenol blue turns yellow,  $\text{NaH}_2\text{PO}_4$  (pH 4.5) is obtained. (Other buffers would have to be removed before determining phosphate by titration first to brom phenol blue and then to phenolphthalein.)

The phosphoric acid in urine comes from the burning of proteins. A meat and cereal diet results mainly in acid urine and a milk and vegetable diet in alkaline urine. The H ions from  $\text{H}_3\text{PO}_4$  of a bread and meat diet keep the urinary tract sterile. Eating  $\text{NH}_4\text{Cl}$  would have the same effect as eating a bread and meat diet because of the formation of urea and HCl from the  $\text{NH}_4\text{Cl}$ . Ten grams or more of  $\text{NH}_4\text{Cl}$  per day are required to acidify urine to pH 4.7. In pyelocystitis the urine should be kept acid for 3 weeks. Urotropine hydrolyzes only in acid urine and its action has not been clearly distinguished from that of the H ions.

Henderson, L. J.: *Ergebnisse Physiol.* 8:254 (1909).

## PART II

### INORGANIC

#### Water.

##### WATER CONTENT OF TISSUES

<i>Percentage</i>	<i>Percentage</i>
Connective tissue.....60	Blood plasma.....90-92
Cartilage.....68-74	Blood erythrocytes...64-65
Bone.....20-35	Blood.....75-82
Muscle.....75	Milk.....88
Gray matter of brain...83-85	Protoplasm.....75-90
White matter of brain...68-73	Mammalian body....66

Water is the principal solvent in biology just as it is in geology. Some of the lower organisms contain as much as 90% water; others above 96% (jellyfish). Organisms with a hard skeleton have a lower percentage of water than those with a soft skeleton. Fatty tissues are low in water although water is necessary in the beginning of the deposition of fat. About 10 liters of water are secreted, as digestive juices, per day, but they are re-absorbed.

Martin Fischer popularized the idea of bound water. It is not clear whether it is water of hydration or some other form of binding of the water.

Some persons have had the idea that the holding of water by colloidal proteins and carbohydrates is similar to osmotic pressure and make calculations of the Donnan equilibrium between leather, for instance, and the tan liquors. If dried gelatin is placed in a saturated solution of sodium chloride, salt will crystallize out. It might then be supposed that the osmotic pressure inside the gelatin is greater than that of a saturated salt solution. This seems hardly probable from measurements of osmotic pressure of protein solutions as compared to those of salt solutions. Some investigators have called this "swelling pressure" rather than osmotic pressure. If it were a case of hydrate water, it would be more in harmony with our chemical ideas. Calculation of the quantity of bound water is made with difficulty, and different investigators are not much in agreement as to the results.

In contradistinction to a piece of gelatin, for instance, a living

cell is surrounded by a waterproof layer, and the outer layer of some cells will not allow even water to pass through as, for instance, on the unfertilized eggs of certain fish. Water passes through the surface layer of some cells slowly, depending upon the difference in osmotic pressure on the two sides of this membrane. This membrane on many cells is semipermeable in the sense that a copper ferrocyanide membrane is semipermeable, allowing water to pass through and preventing the diffusion of certain dissolved substances. As a general rule, where there has been thorough investigation, sodium and potassium do not pass through, but in many cases chloride ions pass through at measurable rates.

Dead cells lose their water by evaporation faster than living cells. This may be due to a combination of two causes. First — dead cells lose their impermeability to certain dissolved substances, exerting osmotic pressure. But this does not explain the high resistance of many cells to evaporation, which is due probably to a relatively poor permeability to water, which is lost on death of the cell. Such membranes on living cells are very thin but on tissues may be much thicker and resistant. The outer layer of an apple is quite resistant to evaporation, and that of a prune is so resistant that it is customary to dip the prune in alkali so that it will dry faster.

The surface of the cell is called the plasma membrane and is concerned in anesthesia. It is supposed that anesthetics stabilize the plasma membrane and make it either less permeable or less easy to make permeable by stimulation. According to this idea, stimulation may consist primarily in increasing the permeability of the plasma membrane. Anesthetics are substances which reduce the surface tension of the membrane surrounding cells and therefore accumulate at the cell surface. They are supposed to be also soluble in the plasma membrane as they may enter the cell.

It may be that the action of anesthetics on the plasma membrane gives rise to the phenomenon of anesthesia. It is this plasma membrane which is concerned in the phenomenon known as cytolysis, or in relation to blood cells, hemolysis. Hemolysis refers to an increase in permeability of the plasma membrane of the red blood cells. There are many substances normally held back by the plasma membrane, among them hemoglobin. Hemoglobin passes out of the cytolized cell and can be seen, leaving the "ghost" of the cell.

Water is lost in the human body by evaporation from the lungs and in the urine and feces. Evaporation from the skin without visible sweating is called insensible perspiration. The human adult may lose from the skin and lungs about 50 grams per hour in weight, which is mainly water but to a small extent carbon as carbon dioxide. Secretion of urine may amount to about the same, giving a total loss of about 100 grams per hour.

If a large amount of carbohydrate is consumed with water, more water is retained in the body than if water solely is consumed. We do not know just how the carbohydrate holds water. The carbohydrate is absorbed from the gut chiefly as glucose and other sugars, and it may be the osmotic pressure of the glucose which holds some water. This glucose may be distributed to the different tissues, for instance the skin, and increase its water content. Most of the sugar thus absorbed, however, is deposited as glycogen, chiefly in the liver and muscles but also in the skin. The amount of glycogen in the other tissues is relatively small.

Usually with the retention of water there is retention of salts. This is often observed in pneumonia as retention of water in the lungs together with salts, and on resolution of the pneumonia both water and salts are excreted by the kidney at the same time. The same is true of the edema of various tissues.

Edema is caused by water being stored in fluid surrounding cells of the body. When a person increases in weight, the first thing stored is water. Therefore water-starvation is a means of reducing which is very practicable in experiments on animals but not on humans, owing to the fact that it is these very persons who are overweight who cannot suppress their thirst and cannot control their water-drinking.

McQuarrie has been able to prevent epileptic attacks by decreasing the intake of water. A coated tongue results from lack of saliva. In fever, evaporation of water is increased and the lack of water in the body causes a drying of the tongue and the coating is the ultimate result, although a fever associated with a feeling of chill may not always be accompanied by increased evaporation from the skin.

Ordinary fever is associated with a hot skin. Some diseases have been treated by artificial fever. The best way to produce such a fever is by diathermy — passing a high-frequency electric current through the living cells of the body, the electrical resistance

of the cells causing them to heat up. Owing to the fact that the plasma membranes of the cells are the most highly resistant portions, they may be injured before the proteins of the body are coagulated by the heat. There are differences in resistance of different parts of the body, and the current lines follow the path of least resistance. Fifteen pounds of water can be lost by diathermy, resulting in dehydration fever, fever due to the fact that there is not enough water in the body to regulate the temperature by evaporation. Before this great loss of water takes place, the diathermy does not raise the body temperature very much. Local diathermy does not raise the interior of the body more than about  $4^{\circ}$  because the blood carries the heat away into the general circulation. Balcar, Sansum, and Woodyatt produced fever as high as  $125.6^{\circ}$  F. in dogs by diuresis through intravenous injection of glucose.

If placed in a room saturated with water at body temperature one would die from fever. A person heats (without evaporation) 1 cu. m. of air about  $\frac{1}{2}^{\circ}$  per minute. This is the cause of death in crowded places like the "Black Hole of Calcutta." The presence of many human beings not only heats the air, but also humidifies it until finally there is no mechanism for the loss of heat.

To dehumidify air, cold water is sprayed through it (air conditioning), the air being saturated with water at this low temperature. The air is warmed up and then becomes relatively dry. When air is saturated with water in the form of snow in northern weather and then heated up to room temperature, it may contain only 10–15% of its total capacity of moisture.

Apparently the glomeruli of the kidney filter out about 800–2,500 cc. per hour, and ordinarily most of this is re-absorbed by the convoluted tubules.

Rowntree showed that edema may be produced by drinking more than 800 cc. of water per hour for long periods, in fact, this may give rise to general water-intoxication when the powers of eliminating water are exceeded. Haldane and Priestley gave 5,500 cc. in 4 hours. Edema is one of the characteristics of diabetes and nephritis. The nephritis may result in impaired capacity of the kidney to eliminate water. Kunstmann drank 10 liters per day for 4 months. NaCl increased in blood serum and was lost from the body.

In diabetes there is a very intense thirst as well as rapid ex-

cretion of water in the urine. The retention of water may be due to the high concentration of sugar in the tissues, and if a heavy dose of insulin is given, sugar rapidly leaves the blood. A good deal of this water passes into the brain when it leaves the blood on loss of the sugar and, according to Greenwald, gives rise to insulin-shock. If the body is dehydrated before giving the insulin, insulin-shock does not occur on giving insulin but death may result if too much insulin is given.

When large quantities of glucose are eaten the volume of blood may increase (fig. 20), making a larger total quantity of water in the blood-vessels. The skin is supposed to increase in water content also.

There may occur congestion of the respiratory sinuses and nose mucous membrane associated with increased water retention. This might possibly be due to the passive action of increase in blood volume. After a considerable period of congestion, filtration of fluid out of the blood may cause edema of the tissues, and edema of the tissues surrounding the opening of the respiratory sinuses may lead to their closure. The blood then absorbs the contained air, causing a vacuum-headache.

There is edema of the skin in eczema. A person with sinusitis or eczema may be more sensitive to water-retention than ordinary persons.

Balcar, Sansum and Woodyatt: *Arch. Internal Med.* 24:116 (1919).

Hatai: *Carnegie Inst. Wash. Pub.* 251:95 (1917).

Kunstmann: *Arch. exptl. Path. Pharmacol.* 170:701 (1933).

Rowntree: *Physiol. Rev.* 2:116 (1922).

**Stimulating and Depressing Ions.** If certain ions in the blood stream are increased in concentration, certain of the muscles at times go into spasmodic contraction, which is called tetany. Such contractions are noted in swimmers and are called cramps. Sometimes a muscle of the eyelid will twitch or a part of the pectoralis muscle of the breast will twitch. These are symptoms of tetany. They may be due to a superabundance of certain ions in the blood, which are therefore called stimulating ions.  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{OH}^-$  are stimulating ions. On the other hand the opposite of tetany may occur, a condition which may be called coma. The breathing may be slower than usual and there may be a tendency toward drowsiness. This may be due to a superabundance of another class of ions, which may be called depressing

ions. They are  $H^+$ ,  $Ca^{++}$ , and  $Mg^{++}$ . For instance, the injection of  $MgCl_2$  solution into the blood stream may give rise to a condition resembling anesthesia. It is not easy to keep the concentration of any of these ions in the blood at abnormal levels for definite periods of time, and the effects of intravenous injection are very transitory in nature.

Sidney Ringer showed that the heart which has been cut out of the body may beat for a long period if it is provided with a solution of certain salts instead of blood. Oxygen must be provided, and this is most easily done with the hearts of cold-blooded animals, for which it is only necessary to dissolve oxygen or air in the salt solution. A fluid containing suitable ions is now called Ringer's fluid. When a heart is bathed (perfused) with Ringer's fluid the strength of the contractions grows less and less (hypodynamic heart) until finally they cannot be observed at all, and yet, with the string galvanometer, it may be shown that a rhythmic process is still taking place in the heart. An increase in the concentration of  $Ca^{++}$  will revive the hypodynamic heart, at least to some extent, although some lipid seems necessary for complete revival. Ringer showed that for strong contractions  $Na^+$ ,  $K^+$ , and  $Ca^{++}$  are necessary.

Ringer studied mainly the force of the contractions, but the ions also affect the rate of the contractions.  $Na^+$ ,  $K^+$ , and  $OH^-$  increase the rate whereas  $H^+$ ,  $Ca^{++}$ , and  $Mg^{++}$  decrease it. Howell showed a relation between  $K^+$  and vagus action, that is to say, when the heart is in the body if we stimulate the vagus nerve the heart-rate is slowed. Also, if we inject  $K^+$  intravenously the heart-rate is slowed. This may be taken to indicate that  $K^+$  stimulates the vagus, because if we cut the heart out of the body, thus severing the vagus nerves, and increase the  $K^+$ , the heart-rate is increased.

If we decrease the heart-rate more and more, it will finally stop; and also if we increase it more and more it will stop. That does not mean that the increase in rate changed to a decrease, for there is a difference in the way the heart stops. A heart-beat is in two phases, first contraction (systole) and then relaxation (diastole). If we decrease the rate by increasing the  $Mg^{++}$  the heart will stop in diastole, but if we increase the rate by increasing the concentration of  $K^+$  the heart will stop in systole. In other words, the depressing ion favors diastole and the stimulating ion favors systole.

When the heart or any other muscle dies it goes into a contraction known as rigor mortis. These ions in pure solution may be rather toxic and lead to death of the heart, but some are more toxic than others.  $\text{Na}^+$  and  $\text{Mg}^{++}$  are the least toxic. If the heart is stopped in diastole by  $\text{Mg}^{++}$ , it remains in diastole a long time, but if the heart is stopped in diastole by  $\text{Ca}^{++}$  it will very probably go into death rigor, in which case it might appear to stop in systole. The two processes cannot be separated except by very minute gradations in the concentration of  $\text{Ca}^{++}$ . This effect, together with the action of  $\text{Ca}^{++}$  in reviving the hypodynamic heart, has led to the error of calling  $\text{Ca}^{++}$  a stimulating ion. On the other hand, the action of  $\text{K}^+$  in stimulating the vagus as well as the very powerful stimulating power of  $\text{K}^+$  on the heart itself, which may result in stoppage in systole, easily give rise to the error of calling  $\text{K}^+$  a depressing ion, but by comparing a large number of observations on different tissues it seems more logical to make the generalization that  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{OH}^-$  are stimulating to motor nerves and increase the rate of the heart-beat whereas  $\text{H}^+$ ,  $\text{Ca}^+$ , and  $\text{Mg}^{++}$  are depressing to motor nerves and decrease the rate of heart-beat.

All these generalizations are not easily applied to special structures, such as the nerve endings in the skin and the respiratory center. Nerve endings in the skin are stimulated by a high concentration of  $\text{H}^+$ , and the respirations are stimulated by increasing the concentration of  $\text{H}^+$ .

Whereas the respiration is automatically affected by H ions it is to a certain extent controlled by the will. By forced breathing one may increase the pH of the blood until tetany results (increase of 0.2 in pH, Peters).

Peters, Bulger, Eisenman, and Lee: *J. Biol. Chem.* 67:175 (1926).

Ringer: *J. Physiol.* 18:425 (1895).

**Lithium** ions may be partly substituted for  $\text{Na}^+$ , and both are stimulating to different degrees. When lithium salts are taken in large amounts they produce nausea, diarrhea, rapid breathing, and convulsions, and death may occur.

Lithium is said to be a solvent for uric acid because lithium urate is more soluble than sodium urate (lithium urate is 0.27% soluble at 20°). Tri-lithium-phosphate is only 0.04% soluble. The blood is not sufficiently alkaline for precipitation of  $\text{Li}_3\text{PO}_4$ .

but it might occur in the bones. Lithium carbonate is given by mouth in doses of 1 g. in an attempt to dissolve the sodium urate out of the joints of gouty persons, but this seems futile. In sea water there are 7 atoms lithium to 1,070,000 atoms sodium (Goldschmidt) or 0.1 mg. per liter (Thomas).

Daniels: Arch. Internal Med. 13:480 (1914).

Goldschmidt, Berman, Hauptman, and Peters: Nach. Ges. Wiss. Göttingen (Math. Phys.) 1933:235.

Thomas and Thompson: Science 77:547 (1933).

**Sodium** is the chief base of the blood plasma. A 1% solution of sodium chloride is called a "physiological salt solution" because it can be injected into the veins without causing any ill effects and is soothing to cuts and wounds and parts left bare by surgery. Only part of the sodium in the blood plasma is in the form of the chloride. Some is in the form of bicarbonate but this does not quite reach 0.03 N (65 vol. %  $\text{CO}_2$ ). Sodium bicarbonate is very important from the standpoint of hydrogen-ion concentration, as we have already mentioned.

There are 335 mg. of sodium in 100 cc. of blood serum (about 150 milli-equivalents per liter) and about the same amount in the spinal fluid, pancreatic juice, and bile, but only 43 mg. per 100 cc. in the blood corpuscles and about 80 mg. per 100 g. muscle. Corpuscles are impermeable to sodium if they are fully formed.

The sodium bicarbonate in plasma has been called *alkali reserve*, since it will neutralize strong acid that may find its way into the

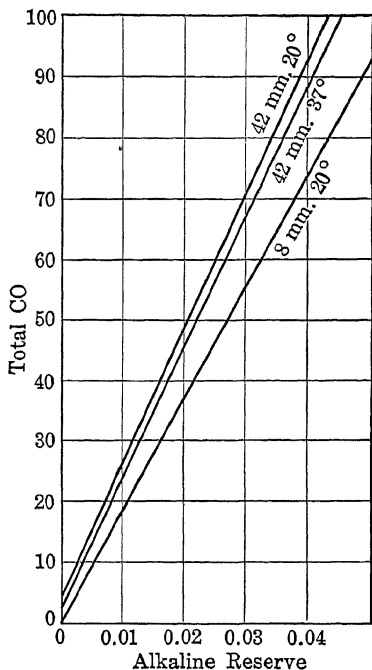


FIG. 25. Alkali reserve and Van Slyke  $\text{CO}_2$  values of blood plasma. Journal of Biological Chemistry.

blood. It may be expressed as alkali or as  $\text{CO}_2$  (fig. 25).  $\beta$ -hydroxybutyric acid may accumulate in the blood and decompose the bicarbonate. This acid is excreted by the kidneys partly in the form of the sodium and partly in the form of the ammonium salt. The excretion of the sodium salt lowers the bicarbonate of the blood. This is often lowered to a dangerous limit in diabetics.

When the bicarbonate is lowered, the  $\text{CO}_2$ -pressure must be lowered to maintain the hydrogen-ion concentration in physiological limits; and in order to lower the  $\text{CO}_2$ -pressure, rapid breathing occurs. When such rapid breathing is seen in diabetics, it is an indication of the lowering of the bicarbonate, and either bicarbonate or food whose ash contains bicarbonate is given.

Since the sodium ion is stimulating, an increase of the bicarbonate may make the nerves easily excited. When one eats 40 g.  $\text{NaHCO}_3$ , this is usually excreted rather rapidly, but if the kidneys are impaired, tetany may result when the alkali reserve reaches 0.04*N*.

Gamble and McIver: J. Exptl. Med. 48:849 (1928).

**Sodium chloride** is very low in concentration in animals except for the blood plasma and other tissue fluids. Plants are rather low in sodium chloride. Hence we put salt on food. Sweat is mainly a sodium chloride solution, being 0.7%  $\text{NaCl}$ . It contains a number of other diffusible substances, however.

One ordinarily excretes about 10 g. of  $\text{NaCl}$  in the urine a day. On starvation the concentration in the urine goes down very much. A man fasting a month at the Carnegie Nutrition Laboratory was excreting 2.5 g. of  $\text{Na}_2\text{O}$  per day at the beginning. On the fifth day this dropped to 0.3 g., and from the tenth day on there was 0.1 g. or less. On giving a diet containing 10 g.  $\text{NaCl}$  a day, it took four days for the excretion to rise above 0.1 g. per day. The body retained it to replenish the deficiency.

The concentration in sweat is constant, and excessive sweating causes stokers' and miners' cramps, owing to the loss of  $\text{NaCl}$ . Stokers on ocean liners work in a room at about  $150^\circ \text{F.}$ , stripped to the waist. They drink water in enormous quantities to replace that lost in sweat. It has been found that unless they are given salt in some form they will develop cramps. The simplest way to do this is to add one-fourth of the quantity of sea water to their drinking water or make it about 0.7% salt.

As early as 1873 Forster found that dogs died on a salt-free diet composed of starch, lard, and meat that had been extracted free of salt. Bunge supposes that this is due to a depression of the bicarbonate of the blood and that when ordinary food which contains much potash, is eaten, the kidney cannot distinguish between potassium and sodium very well and some of the sodium is excreted in getting rid of the potassium.

The sodium of the pancreatic juice is about the same as in blood plasma but the chlorine is largely replaced by  $\text{HCO}_3^-$ .

Baird and Haldane found that 40 g. of NaCl a day caused edema in a normal man. In case of nephritis even smaller amounts are thought to cause edema and hence the salt of the diet may be cut down.

Abderhalden: *Lehrbuch der physiologischen Chemie*, fifth edition, Berlin (1923).

Babcock: *Wis. Ag. Exp. Sta. Ann. Report*, 129 (1905).

**Chloride.** There are about 370 mg. of chloride per 100 cc. blood plasma and about the same in the gastric juice — in fact, there are about 370 mg. HCl in 100 cc. gastric juice (the weight of hydrogen being negligible), which shows that practically all of the chloride in the gastric juice is in the form of HCl, whereas that in the blood plasma is in the form of NaCl. If HCl is vomited from the stomach, the plasma chlorine may be reduced to half the normal.

In nephritis and cardiorenal diseases, the chloride content of the blood is high, probably on account of impaired excretion. Excretion of chlorides is increased by pitressin. When a large volume of urine is excreted, although the salt content may be very much reduced, the total excretion of salt is increased owing to the fact that the urine is never free from salt. If the loss in water is made up by drinking water, the NaCl content of the blood may be reduced as is often true in diabetes (which means an increased flow of urine). In diabetes mellitus 30 g. NaCl has been given in 12 hours without hyperchloremia.

Chlorides of hydrogen, calcium (45 g. per day), and magnesium act in the same way, all being absorbed as HCl;  $\text{NH}_4\text{Cl}$  (20 g. per day) acts similarly owing to the change of ammonia into urea in the liver. Part of the HCl is excreted as NaCl and part as  $\text{NH}_4\text{Cl}$ , but there is usually a lowering of the bicarbonate of the blood.

Foods that are poor in sodium are poor in chlorides, and this includes all foods, except blood, which is seldom used as food.

Many higher mammals go to salt licks for their salt. Some of these salt licks contain considerable bicarbonate but that is not what gives the salt a taste and leads them to the lick.

Denis and Sisson raised the chloride content of the blood plasma up to 432 mg. per 100 cc. by feeding salt. On the other hand, if an animal is fed a diet deficient in chloride, there is finally achlorhydria (lack of HCl in the stomach) which may be detected by gastric analysis. One experiment of Frouin's demonstrated achlorhydria in dogs on an eight-day NaCl-free diet. Achlorhydria occurs in pernicious anemia. The amounts of HCl prescribed by physicians in this disease are very small — in fact, if 0.1 N HCl is drunk by such patients they complain of gastric distress.

**Carbon Dioxide.** Although the bicarbonate in the blood has been much thought about as a mechanism of preserving the neutrality, this may not be its first function. There are other substances — phosphates and proteins — which help to preserve neutrality. An important function of the bicarbonate is the carrying of carbon dioxide from the tissues to the lungs.

When carbon dioxide in the blood exceeds 45 mm. Hg. partial pressure, it stimulates the respiratory center and is exhaled at a faster rate, thus returning to this level, so that the partial pressure of carbon dioxide of the alveolar air in the lungs remains roughly 45 mm. Hg. Since there are 125 sq. m. of surface in the alveoli of the lungs, the arterial blood is at equilibrium with the alveolar air.

When the blood goes through the tissues, more carbon dioxide is added to it. Because the bicarbonate in the plasma cannot take up any more carbon dioxide, some other mechanism is necessary. The carbon dioxide decomposes potassium hemoglobinate and forms potassium bicarbonate in the corpuscles. Owing, however, to the chloride shift, this bicarbonate passes to the plasma. A slight amount of carbon dioxide could be carried without the presence of the corpuscles, owing to the fact that the plasma contains 8% protein, which is partly combined with sodium, and carbon dioxide decomposes the sodium-protein combination, forming sodium bicarbonate directly in the plasma.

Carbonic acid is a buffer, and the point of greatest buffer action is pH of 6.5. It has, however, considerable buffer action at the

pH of the blood. If the blood were a pure bicarbonate solution, the change in the carbon dioxide content between arterial and venous blood would cause a greater pH change than that which actually occurs, which is only 0.03. In other words, the hemoglobin helps buffer the pH of the blood against the stronger carbonic acid.

In carbonate-bicarbonate solutions the respiration of organisms may be studied by the dissociation ( $\alpha$ ) of a suitable indicator (weak acid), such as phenol red or brom thymol blue. For example, in sea water with alkali reserve = 0.0023 *N* the following table gives the change in CO<sub>2</sub> (in cubic centimeters per liter) with change in  $\alpha$ :

$\alpha$	Change in CO <sub>2</sub> (cc. per l. sea water)
0.09	0.705
0.10	4.920
0.20	3.300
0.30	2.675
0.40	2.470
0.50	2.470
0.60	2.675
0.70	3.300
0.80	4.920
0.90	

Van Slyke: *Physiol. Rev.* 1:141 (1921).

Chlorate is not interchangeable with chloride. Although, when eaten, a trace is reduced to chloride in the body, 90–96% may be recovered. If a toxic dose (10 g.) is taken, a considerable amount of oxyhemoglobin is changed to methemoglobin. Most of the methemoglobin passes out the kidneys, whereas some is changed to bile pigments, which are therefore increased in amount. Fifteen grams of KClO<sub>3</sub> may be fatal. Chlorates are used for mouth-washes as well as for killing weeds. They are excreted in the saliva, perspiration, tears, and urine.

Chlorate in certain solutions liberates chlorine gas. It should be emphasized that free chlorine does not cure influenza or the

epidermophyton infection known as athlete's foot but merely sterilizes air and water so as to prevent infection of other persons.

Cameron and Hollenburg: J. Biol. Chem. 44:239 (1920).

**Potassium.** The concentration in blood plasma = 20 mg. per 100 cc. In the cerebrospinal fluid it varies from 15 to 20 mg. Owing to the rapid excretion by the kidneys the change in the intake of potassium does not vary these concentrations very much.

The concentration in the corpuscles is from 400 to 428 mg. per 100 cc. The potassium in the corpuscle cannot escape until the corpuscle is destroyed although it is partly ionized.

The concentration in muscle is 300-400 mg. per 100 g. George Fahr showed that the potassium could not diffuse out of the muscle. When, however, the muscles decrease in size owing to starvation, an equivalent quantity of potassium is lost. Toward the end of a month's fast a man at Carnegie Nutrition Laboratory eliminated 0.75 g.  $K_2O$  per day, but when muscle-building foods were fed, although they contained potassium, the excretion dropped to zero since the increment of muscle tissue required potassium.

The ordinary foods are rich in potassium. White bread contains 0.11%, cow's milk and eggs 0.14%, potatoes 0.43%, and striated muscle 0.32-0.42%. There is, therefore, never a deficiency of potassium on an ordinary diet, nor is there during starvation, since the starving muscles release potassium into the blood. No one has determined the potassium requirement in man. On the other hand, if 25 g.  $KCl$  is taken by mouth, it will cause diarrhea and thus escape absorption.

Owing to the fact that there is a good deal of potassium in the ordinary diet, it being particularly high in meat and potatoes, there is a very ready excretion of it in urine and sweat. The excretion is not very selective, however, as Bunge showed that the kidney could not well distinguish between potassium and sodium (confirmed by Miller). Bunge fed himself 19 g. of potassium citrate and excreted all of the potassium in a short time together with 6 g.  $NaCl$ . Therefore, one has to put  $NaCl$  on potatoes and meat to provide for the loss there would otherwise be during the excretion of the excess potassium.

Potassium is a stimulating ion, but *over-stimulation* is not always properly interpreted. Thus an excess of potassium *stops* the

excised heart, by reason of the fact that it causes a contraction which does not relax and therefore the heart is not prepared for another contraction.  $K^+$  may slow the heart in the body owing to vagus stimulation. Zwaardemaker supposes the physiological action of potassium to be due to its radioactivity and has substituted radium for potassium in certain experiments. Radium is very toxic, however.

Potassium salts added to water aid in putting out oil fires; there is enough of the element in sea water for this purpose. Potassium salts on a lump of sugar aid in its combustion.

Howell: *Am. J. Physiol.* 15:280 (1906).

Macallum: *Arch. neerland. Physiol.* 7:304 (1922).

Zwaardemaker: *Vers. Akad. Wettensch. Amsterdam* 26:776 (1918).

**Rubidium** is a stimulating ion and can be substituted for  $K^+$  in the growth of certain bacteria. In mammals, however,  $Rb^+$  occurs in only small amounts in milk and blood. When it is increased in amount, it stimulates the respiratory center. In sea water there are 2 atoms of rubidium to 107,000 atoms of sodium.

Roffo: *Boll. ist. med. sper.* 2:955 (1926).

Wright and Papish: *Science* 69:78 (1929).

**Cesium** is stimulating and may be substituted for  $K^+$  to a certain extent. Zwaardemaker suggests that it probably emits  $\beta$ -rays. If  $Cs^+$  is substituted for  $K^+$  in the perfusion fluid of a muscle, it will be substituted inside the muscle fibers, when they are contracted (tetanized) but not when they are relaxed. Rats died when half the  $K^+$  in the muscles was replaced by  $Cs^+$ , which occurred after seven to ten days on a diet in which  $K^+$  was substituted by  $Cs^+$ . The content of sea water is about 2 atoms of cesium to 10,700,000 atoms of sodium.

Kaiser: *Arch. Neerland Physiol.* 3:587 (1919).

Walbum: *Z. Immunitats.* 43:433 (1925).

Zwaardemaker: *Vers. Akad. Wettensch. Amsterdam* 26:776 (1918).

**Beryllium** is a depressing ion and may be substituted for two-thirds of the  $Ca^{++}$  in the perfusion fluid of a frog's heart. However, it is toxic when taken by mouth or injected in considerable amounts.

Duliere and DeBorggraef: *Compt. rend. soc. biol.* 98:1255 (1928).

**Magnesium** is depressing and much less toxic than  $\text{Be}^{++}$ . There are about 2-3 mg. per 100 cc. of blood plasma. Some magnesium occurs in the bones and it increases with age. Perhaps it may be secondarily deposited as in the formation of dolomite from limestone. It is so depressing that when injected intravenously it acts as an anesthetic and may paralyze the respiration and heart. Magnesium salts are injected for the relief of tetany caused by the tetanus bacillus or by excessive stimulating ions in the blood, such as the tetany or spasmophilia of infants.  $\text{Mg}^{++}$  seems to be a more efficient depressant than  $\text{Ca}^{++}$ , although  $\text{Ca}^{++}$  is the chief depressing ion of the blood plasma. Joseph and Meltzer showed that the excitability of the nerve-muscle junction in frogs which was paralyzed by  $\text{Mg}^{++}$  could be restored by  $\text{Ca}^{++}$ . It is well known that  $\text{Ca}^{++}$  is a powerful depressant, and one would suppose that the  $\text{Ca}^{++}$  must aid in the elimination of  $\text{Mg}^{++}$ .

It has been found that stimulating ions increase the permeability of the plasma membrane of some living cells and depressing ions decrease the permeability; and  $\text{Ca}^{++}$  aids  $\text{Mg}^{++}$  in decreasing permeability and does not antagonize it. The plasma membranes of different cells and parts of cells, as at nerve-muscle junctions, behave somewhat differently; in fact, their behavior is not at all understood, and this simple relation of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  to a single cell does not hold for the whole body.

It is a fact that  $\text{Ca}^{++}$  injections may save the life of an animal after an overdose of  $\text{Mg}^{++}$  but it has to be given very quickly, otherwise death due to paralysis of the respiration or heart will already have occurred.

$\text{Mg}^{++}$  depresses movements of the gut instead of stimulating them as do many cathartics. It is commonly believed to be absorbed only with extreme slowness especially when given as magnesium sulfate. It increases the bulk of the feces by drawing water into the lumen of the gut as a result of the increased osmotic pressure. Since it is the bulk of the feces distending the rectum which causes the reflex for defecation,  $\text{Mg}^{++}$  is said to be cathartic although it does not stimulate the gut in general as calomel and jalap do — in fact, it has the opposite effect and it may save the life of a patient who has taken an overdose of a cathartic which otherwise would cause a spasm that might continue until necrosis occurred. When epsom salts are given with olive oil, magnesium

oleate is excreted in lumps, which have been mistaken for gall stones.

It is supposed that the application of  $Mg^{++}$  to the duodenum relaxes Oddi's sphincter, which may be followed by evacuation of the gall bladder. Since such an evacuation is not directly caused by  $Mg^{++}$ , such a conclusion does not necessarily follow:

McCollum states that a magnesium-free diet produces marked osteoporosis, the bones casting no X-ray shadow.

Magnesium may be of such high concentration in soils as to be toxic to plants. The beneficial effect of liming such soils may be the increase of  $pH$ , thus making magnesium less soluble. Phosphates may have a beneficial effect in the treatment of such soils by causing the precipitation of  $MgNH_4PO_4$ .

Joseph and Meltzer: *Am. J. Physiol.* 29:1 (1911).

Medes: *Proc. Soc. Exptl. Biol. Med.* 23:496 (1926); *J. Biol. Chem.* 68:296 (1926).

**Elemental phosphorus** exists in yellow, red, and black forms. Red phosphorus is insoluble but contains traces of yellow phosphorus, so that large doses are toxic. The use of phosphorus in cod-liver oil in the treatment of rickets is the result of an idea based on the fact that in phosphorus poisoning the bones are damaged. It was argued that rickets is "inflammation of the bones" and phosphorus causes inflammation of the bones; therefore since like cures like phosphorus should cure rickets. It was dissolved in cod-liver oil to prevent oxidation, which probably explains any curative action it might have had. It should be considered merely as a poison. The best antidote is copper sulfate, 0.1–0.2 g. every two minutes until vomiting results. Also the particles of phosphorus are coated with metallic copper. If it is oxidized to  $P_2O_5$  and hydrated to  $H_3PO_4$  it is non-toxic.

Weinstock and Hess: *Am. J. Diseases Children* 32:483 (1926).

**Calcium and Phosphate.** The adult body contains about 700 g. phosphorus (as  $H_3PO_4$ ). There is about 10 mg. calcium and 4.5 mg. acid-soluble phosphorus per 100 cc. blood plasma. Only one-half of the calcium is diffusible and one-fourth is ionized. Since there is only about 2 mg. phosphorus per 100 cc. cerebrospinal fluid, it would not appear as though all the acid-soluble phosphorus of the blood plasma were diffusible, the spinal fluid

being in diffusion equilibrium with the blood plasma in respect to several of its constituents.

Ninety per cent of the phosphorus and 99% of the calcium of the body occur in the bones and teeth. X-ray spectrographs and chemical studies indicate that  $\text{Ca}_3(\text{PO}_4)_2$  does not exist, possibly on account of the fact that the crystal-lattice needs other atoms to complete it. It is probable that the mineral in the bones and teeth is a mixture of fluorapatite,  $\text{CaF}_2[\text{Ca}_3(\text{PO}_4)_2]_3$ , chlorapatite,  $\text{CaCl}_2[\text{Ca}_3(\text{PO}_4)_2]_3$ ,  $\text{Ca}(\text{OH})_2[\text{Ca}_3(\text{PO}_4)_2]_3$ , and podolite,  $\text{CaCO}_3[\text{Ca}_3(\text{PO}_4)_2]_3$  or partly as a mixed apatite known as francolite,  $\text{CaF}_2\text{CaCO}_3[\text{Ca}_3(\text{PO}_4)_2]_3 \cdot \text{H}_2\text{O}$  which occurs as a mineral and probably occurs to a large extent in the bones and teeth, particularly the enamel of the teeth.

Shear supposes that the first precipitation in the bones is brushite,  $\text{CaHPO}_4$ , because  $\text{Ca}^{++}$  and  $\text{HPO}^{--}$  are more concentrated in the blood than any other ions containing calcium or phosphorus or both. Since brushite has been shown not to occur in bones and teeth, we must overlook this suggestion. Robison pictures bone formation as the precipitation of calcium phosphate following the hydrolysis of calcium-glucose-phosphate by phosphatase which is more abundant in regions of bone formation than elsewhere.

The formation of bone occurs in two stages, the first being the formation of the gelatin-yielding substance (collagen — sometimes called ossein), which is called osteoid tissue, and the second stage being the mineralization of the ossein. The clinical observation of the over-production of osteoid tissue without mineralization (or the demineralization of the bone leaving the ossein) has attracted a great deal of attention and is popularly known under the name of rickets. Rickets may be induced in pups and rats and occurs in young animals in zoological gardens. It has never been shown, however, that rickets occur in chickens, for instance, but these have been widely used as experimental animals in this line of work in laboratories where the pathology of rickets is not understood.

From the standpoint of nutrition of the growing mammal, perhaps the most important fact is that lack of calcium in the diet stops the growth (fig. 26). Therefore, the clinical picture obtained from the lack of calcium in the diet may be very different from that obtained from the lack of phosphorus (fig. 27). When

it comes to a baby, however, the diet may not be the same from day to day and at one time the lack may be chiefly calcium and

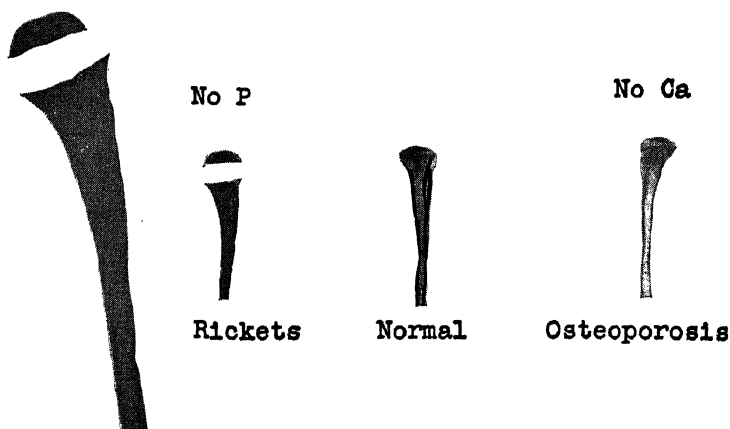


FIG. 26. X-ray positives of head of the tibia of rats of the same litter and on the same diet lacking vitamin D, except that the one on the left lacked P also and developed rickets with a wide space (osteoid tissue) between the epiphysis and diaphysis and that the one on the right lacked calcium and developed osteoporosis with thin wall shaft and large marrow cavity. An enlargement of the one on the left is shown also.

another time chiefly phosphorus. Some confusion then arises by calling this variable condition rickets, that is to say, one might have intermittent rickets or developing rickets or healing rickets, which are not always separated clinically.

In our experiments *rickets have never been produced except with a deficiency of phosphorus in the diet*. We have never produced rickets as a clinical picture with a deficiency of calcium; we explain this as due to the fact that calcium stops the growth of the osteoid tissue and therefore prevents the formation of rickets by producing osteoporosis instead.

A meat diet may be low in calcium but not in phosphorus. Very few meat-eaters, Eskimo and Sioux, had dental caries.

Ortho-phosphoric acid passes over into pyrophosphoric acid at



FIG. 27. Rickets in a pup. Committee on Accessory Food Factors, Sp. Rpt. No. 38.

250°. Pyrophosphates are somewhat stable but the free acid passes over to ortho-phosphoric in water solution, even at room temperature. It has been supposed that pyrophosphates exist in living tissue because on making them acid there is a gradual increase in ortho-phosphoric acid. This is probably due, however, to hydrolysis of a compound of adenylic acid with two phosphoric acid molecules. (Free adenylic acid is composed of adenine, ribose, and one phosphoric acid molecule.) In muscle there are 45 mg. phosphorus as adenylic acid diphosphate per 100 g. and 5 mg. phosphorus as glucose-mono-phosphate and 65 mg. as phosphocreatine.

If muscle pulp is poisoned with sodium fluoride or iodo-acetic acid, glucose diphosphate is formed and the phosphocreatine is hydrolyzed.

There are 20 mg. phosphorus as inorganic phosphate in 100 g. of muscle. It seems probable that some of the adenylic acid may change to inosinic acid. Stella claims that only 8-10 mg. of the inorganic phosphorus is diffusible.

In 1928 Eggleton observed a labile compound which he believed was glucose phosphate in the muscle and called it *phosphagen*. Fisk and Subbarow showed that this was *phosphocreatine*. Harden and Young had already observed glucose phosphate in yeast juice in 1900. Embden postulated that hexose phosphate is hydrolyzed during muscular contraction and called it *lactacidogen*.

Recent investigators claim that lactacidogen is increased during muscular contraction and adenylic acid diphosphate is hydrolyzed. In stimulating muscle to complete fatigue, ammonia is produced, which is believed to be removed from adenylic acid, changing it to inosinic acid. Lohmann claims to have isolated barium pyrophosphate from a trichloroacetic acid extract of muscle. Davenport and Sacks, 1929, could find no free pyrophosphate in muscle, and Lohmann claims that it is bound with adenylic acid.

Eggleton and Eggleton: J. Physiol. 63:155 (1927).

Embden: Z. physiol. Chem. 179:149 (1928).

— and Zimmerman: Z. physiol. Chem. 167:114 (1927).

Fiske and Subbarow: Science 65:401 (1927).

Kramer and Shear: J. Biol. Chem. 79:147 (1928).

McClendon: Am. J. Physiol. 61:373 (1922).

McCollum, Simmonds, Shipley and Park: Bull. Johns Hopkins Hosp. 33:31 (1922).

Mellanby: Physiol. Rev. 8:545 (1928).

Park: *Physiol. Rev.* 3:106 (1923).

Sherman and Pappenheimer: *Proc. Soc. Exptl. Biol. Med.* 18:193 (1921).

Steenbock, Black, and Thomas: *Ind. Eng. Chem.* 19:906 (1927).

Taylor and Sheard: *J. Biol. Chem.* 81:479 (1929).

**Osteoporosis** (fig. 26) and **tetany** are due to deficiency in calcium. If  $\text{CaCl}_2$  is taken by mouth, some calcium goes out in the feces and  $\text{HCl}$  in the urine. This relieves tetany, not by raising blood calcium but by increasing blood  $\text{H}^+$ . The order from greatest to least absorbability of calcium salts is carbonate, sulfate, acetate, bromide, lactate, and phosphate. The presence of vitamin D (viosterol) results in more calcium retained in body and less passing out in the feces, but it is not known that viosterol increases absorption. A fasting man excretes calcium in the gut. Bergeim claims that calcium is absorbed in the upper part of the gut (duodenum) and excreted in the ileum and colon. Fat and bile are said to be necessary for calcium absorption. It has been claimed that infants absorb a higher percentage of calcium from human milk than from cow's milk, but this is probably a difference in excretion on a higher intake.

Viosterol may raise the calcium-ion concentration in the blood to some extent. The hormone of parathyroid, parathormone, raises the blood calcium enormously and leads to its excretion. If a sufficient amount of parathormone is given, the bones dissolve.

Osteoporosis is sometimes caused by lack of vitamin B.

Without vitamin C, dentine is not secreted in the teeth and osteoporosis of the bones results. This condition is called scurvy.

Two per cent of the body weight is calcium. Three-fourths gram per day is probably necessary in the diet (Sherman). Less is necessary when receiving vitamin D.

$\text{Ca}^{++}$  depresses irritability of nerves and muscles, and hence low blood calcium is found in tetany.

The hypodynamic heart (heart perfused with salt solution until its beat is very weak) is revived by  $\text{Ca}^{++}$  and an unknown lipid. The early work on the action of ions on the heart was done by Sydney Ringer. When the heart is perfused with Ringer's fluid (salt solution developed by Ringer), it will continue to beat outside of the body. The fluid contains essentially sodium, potassium, and calcium ions. Sea water is a good perfusion fluid for the heart of *Strombus*, the conch or large sea-snail. If the

concentration of calcium is increased sufficiently, the heart is slowed, but if excessive it will go into rigor mortis owing to the toxicity of calcium.

If certain fish eggs are put in water, none of the electrolytes can come out. If injured slightly by unbalanced salt solution ( $\text{NaNO}_3$ ),  $\text{Cl}^-$  diffuses out and may be detected by the nephelometer. Calcium ions and anesthetics prevent  $\text{Cl}^-$  from coming out.

Delage showed that, if calcium is taken out of sea water, the cells of marine animals fall apart, which is of interest in relation to scurvy, in which disease calcium is excreted by the kidney and the endothelial cells of the capillaries fall apart. Wolbach showed that to retain the cohesive properties of cells it is necessary to have vitamin C and  $\text{Ca}^{++}$ . E. V. Wilson repeated the work of Delage. He took a small sponge and put it in calcium-free sea water and the cells fell apart. When calcium was put back in the water, the cells grew together again to form smaller sponges.

If the kidney is perfused with Ringer-Locke fluid, it will secrete normal urine. If calcium is removed, glycosuria results.

As previously mentioned, phosphates occur as esters with glycerol, glucose, and other substances in the body. It is stated that kidney phosphatase controls the excretion of phosphate. The phosphatase of the blood has an optimum activity with a  $\text{pH}$  of about 6. Phosphates (as well as calcium) are absorbed in the upper portion of the small intestine; the amount absorbed may depend on the ratio of phosphorus to calcium but also on the length of time the contents remain in the upper portion of the gut, and absorption is, therefore, lessened by diarrhea. Organic phosphates are hydrolyzed in the digestive tract. On drinking alkali the excretion of phosphate (and calcium) occurs chiefly in the lower gut; on drinking acid the excretion is largely diverted to the urine.

Phosphates control the  $\text{pH}$  of urine and exist there as the mono and di salts. When all the phosphate is in the form of the di salt, however, the amount in the urine is extremely small and most of it passes out with the feces. Usually the urine is slightly acid and contains about 60% of the phosphate whereas 40% goes out with the feces. In diuresis the proportion in the urine increases, and in diarrhea the proportion in the feces increases. Also an increase in the calcium-phosphorus ratio in the diet increases the

amount in the feces. The excretion is increased with cell destruction, such as resolution of pneumonia.

Phosphates are said to be necessary for the action of salivary amylase.

When phosphates are injected into the blood, calcium and magnesium are decreased, and so phosphates are sometimes injected as an antidote for magnesium narcosis.

The determination of blood serum phosphate is usually divided into the acid-soluble, which includes the inorganic and the hydrolyzable esters (glucose phosphate, phosphoglyceric acid), and the lipid phosphate, which includes esters not easily hydrolyzed, such as occur in phosphatides. Much of the acid-soluble phosphate is in the plasma and the lipid in the corpuscles.

There is a seasonal variation in the phosphate content of the blood, it being highest in June and July, probably owing to the direct effect of sunlight (which irradiates the ergosterol in the skin) and also the eating of food high in vitamin D.

Phosphates are retained in nephritis.

Tetany may be produced by cutting out the parathyroid glands. In this case there is a decrease in calcium to less than 7 mg. per 100 cc. plasma and an increase in phosphate of the blood plasma. Injecting parathyroid hormone increases the calcium and decreases the phosphate in the plasma by excretion of phosphorus in urine. The normal phosphorus content of the cerebrospinal fluid is 1.25–2 mg. per 100 cc., which would indicate that not all the acid-soluble phosphate in the blood plasma is diffusible.

Phosphate may be given to relieve rickets and osteomalacia. Usually, however, they are treated with vitamin D alone on the assumption that there is sufficient phosphate intake if it is used economically. Some milk should be included in the diet in order to insure enough of both calcium and phosphate. Muscle (as a food) is adequate in phosphate and low in calcium, but infants do not eat enough meat to give them adequate phosphate in the diet.

On a meat diet the teeth do not develop properly unless bone, bone-marrow, or other food adequate in calcium content is eaten. The nursing Eskimo receives calcium in its mother's milk.

Hypercalcemia is found in encephalitis, diabetes, and hyperparathyroidism.

Hypocalcemia is found in tuberculosis, asthma, essential hyper-

tension, Addison's disease. parathyroidectomy, castration, and menopause.

Injected  $\text{Ca}^{++}$  disappears rapidly from the blood stream, but while it remains it slows the heart (lowest observed 42 beats per minute), favors strong contractions, contracts peripheral vessels, increases colloid osmotic pressure of plasma, protects capillaries against bleeding, and shortens coagulation time.

It is used clinically as the gluconate to combat hemorrhage, tetany, asthma, lead poisoning, phosgene poisoning, tuberculosis, colitis, and nephritis.

Baráth: Die experimentellen und klinischen Grundlagen der Therapie mit Calciumsalzen, Budapest (1931).

Forbes: Bibliography of Phosphorus Compounds. Ohio Agr. Expt. Sta. Tech. Bull. No. 5 (1914).

Mendel: Nutrition—The Chemistry of Life, Yale Univ. Press, New Haven (1923).

Sherman: Chemistry of Foods and Nutrition, Macmillan, New York (1928).

**Strontium** is similar to  $\text{Ca}^{++}$  in being a depressing ion. It exists in bone and teeth in small amounts and may be determined by its spectrum. It relieves tetany on intravenous injection. According to Shipley and McCollum, 2.2%  $\text{SrCO}_3$  substituted for  $\text{CaCO}_3$  in the diet produces rickets. It has been found in cow's milk. It is excreted chiefly in the intestine as is calcium. It is more depressant than calcium as shown by the fact that the frog's heart when placed in Ringer's fluid in which calcium has been replaced by strontium comes to a standstill. Wheeler found that strontium is capable of replacing considerable of the calcium in the eggshell and bone, and the bones were heavier. Hamburger and Arous observed that strontium can replace calcium in Ringer's fluid perfusing the kidney for preventing the escape of glucose.

Hamburger: Bull. Johns Hopkins Hosp. 34:173, 226, 266 (1923); Biochem. Z. 94:129 (1919).

Shipley, Park, McCollum, and Simmonds: Bull. Johns Hopkins Hosp. 75:378 (1922).

**Barium** is a depressant ion (Scaffidi) and is more toxic than strontium, 6.5 g.  $\text{BaCl}_2$  per kg. being fatal and 0.1 g. per kg. body weight being considered dangerous. In fact, this dose of  $\text{BaCl}_2$  is fatal when injected into dogs, and sometimes half this dose is fatal on injection into rats. Alsberg and Black thought

that loco-weed disease is barium poisoning. This is a disease of stock which have eaten this weed (which contains barium).

Barium sulfate was called in former pharmacopeias *Barium sulfuricum* and is very insoluble and is given to patients to cast X-ray shadows. It is sometimes confused with *Barium sulfuratum*, BaS, which is very soluble in the gastric juice, and this mistake usually leads to death. Sabbatini used sodium sulfate as an antidote, causing precipitation of barium in the gastric juice.

As small a dose as 1 mg. Ba per kg. slows the dog's heart when injected. McGuigan and Ets found barium sulfide fatal when placed on the ears of rabbits. Alsberg and Black were able to replace some of the calcium by barium in the tissues of growing rats. Barium can apparently revive the hypodynamic heart and substitute calcium in the Ringer-Locke fluid for perfusing the kidney and preventing the loss of sugar (Hamburger).

Joseph and Meltzer claimed that barium will antagonize magnesium in producing respiratory paralysis. It is also claimed that barium stimulates the peristalses of the gut, and it is used as a cathartic by veterinarians. It also stimulates uterine and other smooth muscles, whereas magnesium inhibits these. Hanzlik claims that barium chloride acts as a hemostatic.

Alsberg: Proc. Soc. Exptl. Biol. Med. 9:37 (1912).

Hamburger and Alous: Biochem. Z. 94:129 (1919).

Joseph and Meltzer: Proc. Soc. Exptl. Biol. Med. 7:28 (1910).

McGuigan and Ets: J. Pharmacol., Proc. 31:223 (1927).

Scaffidi: Biochem. Z. 9:489 (1909).

**Boric acid**,  $H_3BO_3$ , occurs in traces in the body. Three grams has been found fatal to an infant; 5 g. is toxic to adults (McNally and Rust). The effect is cumulative. Liquor antisepticus contains 2% but 0.2% checks putrefaction in food. Dogs may be poisoned by using it as a dusting powder for wounds (Charmoy). It is rapidly absorbed both from the skin and alimentary tract and appears in the urine 50 seconds after immersing the feet in a hot saturated solution (Kahlenberg). It is used as a hydrogen-ion buffer, when mixed with definite proportions of borax,  $Na_2B_4O_7 \cdot 10H_2O$  (Palitzsch). Moberg and Harding found 0.4 millimole of boric acid per liter of sea water or 20% of the weak acids and hence of significance in determining the pH of sea water.

Charmoy: Pharm. J. 91:649 (1914).

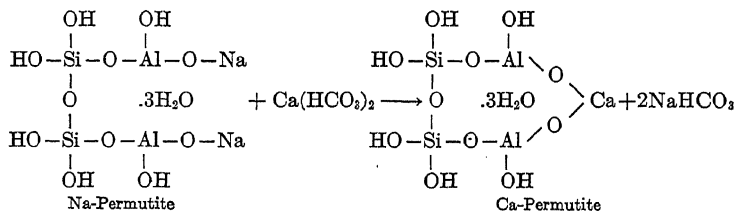
Kahlenberg and Barwasser: J. Biol. Chem. 79:405 (1928).

Moberg and Harding: *Science* 77:510 (1933).  
 McNally and Rust: *J. Am. Med. Assoc.* 9:382 (1928).  
 Palitzsch: *Compt. rend. Lab. Carlsberg* 10:85 (1911).  
 Williams: *Eng. Mining J.* 110:671 (1920).

**Aluminum.** There is 0.2 mg. of aluminum per 100 cc. blood, but 5–8 g.  $\text{AlCl}_3$  per kg. is fatal to animals. It is a powerful depressant and when applied to the skin prevents perspiration by depressing the sweat glands. Soils are very rich in it, but, between  $\text{pH}$  of 4.7 and 8, aluminum forms relatively insoluble phosphates and oxides and is not toxic to plants. Above  $\text{pH}$  8 soluble aluminates are formed, such as  $\text{Na}_2\text{Al}_2\text{O}_4$ , and plants suffer from aluminum poisoning. Also below  $\text{pH}$  4.7, aluminum salts, such as  $\text{AlCl}_3$ , are toxic in the soil.

There has been a controversy over using baking-powder containing alum,  $\text{Na}_2\text{SO}_4\text{Al}_2(\text{SO}_4)_3 \cdot 24\text{H}_2\text{O}$ . It has been used, however, in the treatment of diarrhea. Lamb adds traces of aluminum to synthetic experimental diets.

Aluminum exists in the earth as Fuller's earth (Lloyd's reagent) and zeolites. Permutite is an artificial zeolite,



and is used in water softening and in the laboratory to remove ammonia. The softened water contains sodium bicarbonate, and the permutite is regenerated by leaching with sodium chloride solution.

Greisheimer: *Am. J. Physiol.* 75:366 (1926).  
 McCollum, Rask and Becker: *J. Biol. Chem.* 77:753 (1928).  
 Underhill: *Am. J. Physiol.* 90:15, 52, 72, 76 (1929).

**Lanthanum** is said to replace magnesium in the nutrient solution for bacteria. It acts as a catalyst in ashing biological material (Rask).

Frouin: *Compt. rend. soc. biol.* 68:315 (1910); 72:1034 (1913); *Compt. rend.* 159:410 (1924).

Rask: Personal communication.

**Fluoride** exists up to 0.6% in the enamel of the teeth, which might be taken as evidence that 50% of the enamel is francolite. The average lethal single dose of sodium fluoride for mammals is 500 mg. per kg. by mouth and 150 mg. per kg. subcutaneously. Men have been poisoned by 250 mg. per kg.

The toxicity may be due to the precipitation of calcium. Rats in whose diet fluorapatite was substituted for calcium phosphate developed normally, but fluorapatite produces bad teeth in cows. Smith, Lantz, and Smith showed that fluorine produced mottled teeth in rats and dogs, and that humans with mottled teeth drank water high in fluorine.

Four per cent sodium fluoride will prevent coagulation of the blood.

Large amounts of fluoride cause inflammation of the intestinal tract. It is strongly antiseptic. A concentration of 1:200 prevents growth of bacteria.  $\text{NH}_4\text{F} \cdot \text{HF}$  in 23% solution has been injected around loose teeth. Sodium fluoride is used as a poison for rats and roaches and chicken lice, but sodium silicofluoride is much more effective.

Fluorine is the most common halogen on the earth's surface. Calcium fluoride is very poorly soluble but sufficiently soluble to produce mottled teeth.

Armstrong: Thesis, University of Minnesota (1932).

**Bromide** is less abundant than chloride in the earth's crust. There is 0.3–1.4 mg. per 100 g. of body tissue, blood containing 1–1.5 mg. per 100 cc. When bromides are given in large quantities, the kidney cannot distinguish them from chlorides so that a mixture of chloride and bromide is excreted, thus reducing the chloride and increasing the bromide in the body. A dose of 4 g. of NaBr affects certain parts of the central nervous system and it is thought that the irritability is lessened; at any rate, the respiration is slowed and various reflexes are depressed and drowsiness may appear. The basal metabolic rate is lowered. Ten to 15 g. may cause paralysis.

Large amounts produce edema in the same way that chlorides do, although chloride is less effective in this regard, and the stomach secretes hydrobromic acid. The antidote for bromide poisoning is intravenous injection of 1% NaCl, causing bromide to be eliminated along with chloride by the kidneys.

There are nearly 2 g. bromide per kg. total solids in sea water, and it is extracted commercially by a ship fitted for this operation. Gorgonin, a protein of sea fans, contains  $\frac{1}{4}$ -4%, probably as dibromotyrosine (Mörner).

Cameron and Walton: Trans. Roy. Soc. Canada 22:1 (1928).

Mörner: Z. physik. Chem. 51:32 (1907); 55:77, 223 (1908).

**Iodide.** It has been reported that iodine preparations (thyroid) have been used in medicine for 1400 years, but the element iodine was discovered by Courtois in 1811. Iodide is very much rarer than bromide in the earth's crust — in fact, it is extremely rare but it is distributed very widely and, in fact, occurs everywhere except in filtered air. Many analyses show iodide in the air, but it is in dust particles or in the dust brought down by raindrops. Gautier in France found no iodide or iodine in filtered air. We have confirmed this result many times in this laboratory, even in the winter when every chimney is discharging iodine into the air. It seems that all of this iodine is adsorbed by the soot, and if the soot and other dust are filtered from the air, no iodine can be detected. Von Fellenberg reports iodine in the air, but he absorbed it on cotton moistened with alkali and it is well known that cotton contains iodine. Furthermore, the quantity reported by von Fellenberg is not of medical significance.

It has been supposed that iodine occurs in the rocks in the ratio of the partition coefficient of iodine between these chemical substances, and the ratio of iodine in iron from blast furnaces to the iodine of the slag has been taken as the ratio of the iodine in the steel core of the earth to the iodine in the silicious covering. It seems possible, however, that iodine may have arisen *in situ* by transmutation of elements. The occurrence of helium has been taken as evidence of transmutation of elements in the earth's crust, but iodine, according to Harkins, arises from the expulsion of hydrogen and not helium from heavier elements.

It would seem that the special distribution of iodide as we now find it might be due largely to the fact that organisms absorb and concentrate iodide. This is particularly true of seaweed, which, according to Gautier, absorbs quantitatively all of the iodide out of the surface layers of the sea water. *Laminaria* may contain iodine equivalent to 0.5% of the dry weight. Organisms in the soil absorb iodide, and it is found that the soil often contains

more iodide than the rocks, the weathering of which forms the soil. It would appear as though with the weathering of rocks, sodium and calcium bicarbonates and chlorides are washed away, whereas the iodides are retained by the organisms, and when these organisms die it is absorbed by other organisms in the soil.

Glaciated regions have a low amount of iodide in the soil. This might be due to the fact that the glaciers rubbed off the ancient soil with all its organisms and that the present soil is relatively new — that is to say, it has arisen from the weathering of rock fragments in more recent times than soils of other regions.

There is a small amount of iodide in sea water (estimated at about 25 parts per billion). Therefore there is iodide in rain water near the sea owing to the washing down of salt dust formed by the evaporation of spray, but the amount is hardly significant at a distance greater than 5 miles from the coast.

There are certain deposits high in iodide, such as salt deposits due to the evaporation of ancient seas. In the United States the largest one is due to the evaporation of a gulf which was connected with the Pacific Ocean and covered New Mexico, West Texas, and Kansas, extending even into South Dakota. This was cut off from the Pacific by the formation of the Rocky Mountains and on evaporation produced salt deposits. Certain strata rich in potash are now being mined as a source of potassium. This whole region has artesian water relatively high in iodide.

Other deposits appear which have arisen from the deposition of marine animal or vegetable remains. The principal one is in Chile; it is known as "caliche" and is very high in sodium nitrate and iodate. Since calcium iodate is only about 0.25 per cent soluble in water, it may under certain conditions begin to precipitate from a solution of caliche.

Iodide in rocks varies considerably but it may be as high as 500–1000 parts per billion in granite.

Remington showed that potatoes grown on sandy soil had very little iodide. On the other hand, quartzite and sand may be unusually high in iodide. Iodide in soils may run up to 10,000 parts per billion. Remington showed that potatoes grown on colloidal clay soils are higher in iodide than those grown on sandy soils. It is well known that organic matter is oxidized very rapidly in sandy soil, and it seems possible that the iodide is not held by the inorganic colloids but by the organic colloids and organisms.

Potatoes are very low in iodine when compared with leafy vegetables grown on the same soil.

The iodide in fresh water should bear some relation to the iodide content of mineral deposits or soils with which it comes in contact. There are certain mineral springs in southern California high in iodine and nitrate, which suggests a relationship to conditions in Chile (caliche), and it seems probable that the nitrate and iodine are due to the decomposition of marine organisms or to guano, the latter being the excreta of birds who eat marine organisms. Many artesian waters that are relatively high in iodide are relatively high in salt and some of these have been abandoned for surface water as soon as the chlorination process was made available.

The surface waters of the United States may be divided into two groups (fig. 32) — those from the glaciated regions of the north are very low in iodide (Lake Superior contains 0.01 parts per billion), whereas waters of the south are very much higher, many containing around 5 parts per billion. In the Rocky Mountain region the low iodide content of the water extends further south, and in Utah only 3 out of 17 water samples had more than one part per billion.

Associated with the low or high iodide content of surface water, there is a low or high iodide content of the crops grown on the soil. Barrett showed that the eastern section of Minnesota produced potatoes lower in iodide than those of the western section. Some of the waters lowest in iodide were from the eastern section (for instance, Lake Superior contained only 0.01 parts per billion), whereas the Red River on the west had 0.08 parts per billion.

Besides inorganic iodide, iodine has been shown to occur in organisms as two iodized amino acids combined in protein. Gorgonians (sea fans) contain gorgonin, a protein which on hydrolysis yields diiodotyrosine. The skeleton of the bath sponge is spongin, a protein which yields the same amino acid.

Baumann in 1895 demonstrated that the thyroid (fig. 28) contains iodine, and this was later shown to occur in a protein, thyroglobulin. Kendall at Rochester, Minnesota, in 1914 showed that the iodine was contained in an amino acid, thyroxine, which was obtained by alkaline hydrolysis. When thyroxine is injected, it raises the basal metabolic rate, that is to say, it causes an increased oxidation of foodstuffs in the resting body.

If thyroglobulin is eaten, it is probably broken down in the gut, liberating thyroxine. If it is injected, one encounters difficulties due to anaphylaxis or a sensitization to protein. If elemental iodine is eaten it immediately combines with proteins, and it may be that diiodotyrosine and thyroxine could be formed more easily from elemental iodine than from iodide. On the other hand, iodide is very easily oxidized to iodine.



FIG. 28. Japanese woman with nodular goiter (adenoma of the thyroid). T. Imai.

There is no quantitative method for determining thyroxine or thyroglobulin in the body and no qualitative chemical test to show traces of it. If a large quantity of thyroxine is injected intravenously and the animal is killed immediately, no thyroxine can be isolated from any of the organs by methods at present known. The only way to indicate its presence is by the biological test (tadpole metamorphosis), which does not absolutely differentiate between thyroxine and some other compounds of iodine.

Besides raising the basal metabolic rate, thyroxine causes increased differentiation in the growth processes. If a tadpole is given thyroxine, it will quickly change to a frog. Likewise, if a crystal of elemental iodine is sewed up inside the tissues of a tadpole it will metamorphose to a frog, or if the tadpole is fed on the tissues of an animal that had been injected with heavy doses of thyroxine it will change to a frog. This test, devised by Gudernatsch, has been used to show that the normal transformation



FIG. 29. Cretins of Canton Appenzell, Switzerland.

of the tadpole to the frog is due to the production or accumulation and liberation of thyroglobulin in the tadpole.

Besides the thyroid gland the pituitary gland is rich in iodine, and it has been shown that the pituitary gland releases some substance which stimulates the thyroid gland to cause metamorphosis. If the pituitary gland is removed, no metamorphosis occurs

although the thyroid remains. If, however, both the pituitary and thyroid glands are removed and the tadpole eats the thyroid gland of any mammal, it will immediately metamorphose.

The axolotl is the tadpole of a tiger salamander living in lakes in Mexico that have too little iodine to produce thyroxine for metamorphosis. These axolotls were first thought to be of a different species, but when carried to other countries where there was more iodine, they metamorphosed into tiger salamanders.

In contradistinction to the effects of an increased amount of thyroxine its absence stops development in all vertebrates; thus a human being entirely lacking thyroxine has a basal metabolic rate only half that of the normal, does not develop physiologically, and remains an idiot, being known as a cretin, fig. 29. Cretinism would necessarily result if the individual was absolutely denied iodine from the time of conception, since 65% of the weight of thyroxine is iodine. If, on the other hand, iodine is

# IODIDE

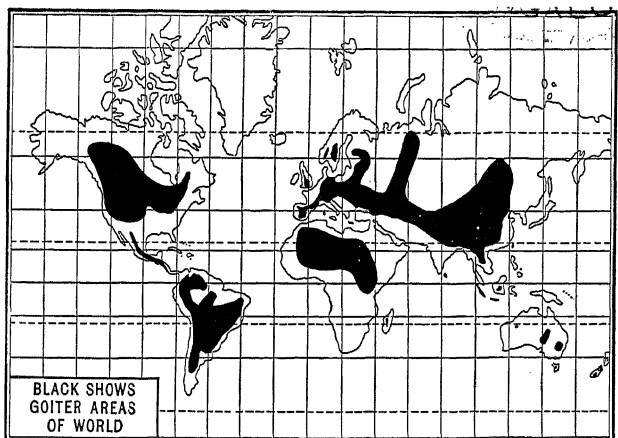


FIG. 30. Rough division between more and less goiterous regions. The interiors of Madagascar, Tasmania, New Zealand, Java, Sumatra, Borneo, Ceylon, Formosa, England and some other islands are goiterous.

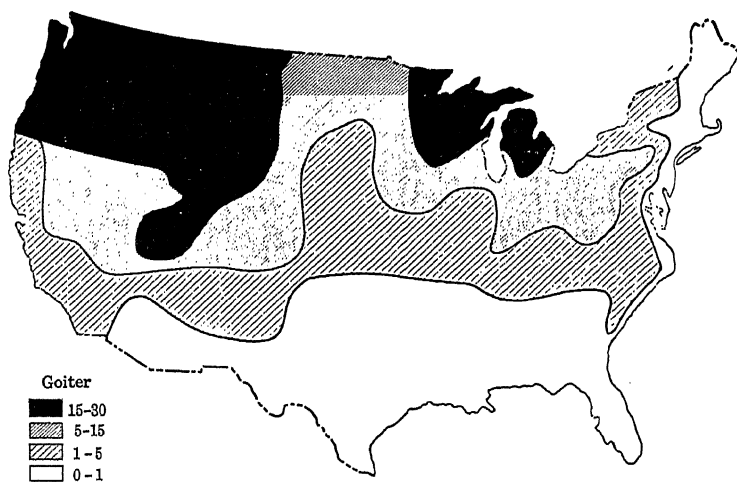


FIG. 31. Simple goiter in the United States per 1000 drafted men. Journal of the American Medical Association.

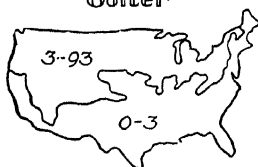
not absent but merely slightly deficient, the thyroid gland begins to grow—that is to say, the cells multiply (hyperplasia). If, at any time during this rapid growth, sufficient iodine is given,

## Exophthalmic Goiter and Iodine in Drinking Water

### EXOPHTHALMIC GOITER

*In the lower area, from 0 to 3 per 1000 drafted men were reported as having exophthalmic goiter ~ in the upper area, from 3 to 93*

### Exophthalmic Goiter



### Simple Goiter



### SIMPLE GOITER

*In the lower area there were from 0 to 5 military goiters per 1000 drafted men ~ in the upper area, from 5 to 111*

### ↑ IODINE in DRINKING WATER

*In the upper area, waters contain from 0.01 to 0.22 parts of iodine per billion parts of water ~ ~ ~ in the area below, from 0.23 to 165*

### Iodine

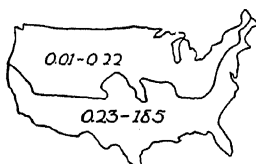


FIG. 32. A comparison of these charts shows that the rate of exophthalmic goiter is high where simple goiter is high and iodine is low and exophthalmic goiter is low where simple goiter is low and iodine is high.

the cells stop multiplying and secrete thyroglobulin, which is called "colloid" by histologists. The increased growth of the thyroid is called goiter, figs. 28, 30, 49, 50.

The increased growth of the thyroid is due to lack of thyroxine and can be stopped at any time by injecting thyroxine; in fact,

the normal growth of the thyroid may be inhibited by injecting thyroxine. After abnormal growth takes place, if the thyroid acquires iodine, thyroxine is produced and yet the thyroid may not shrink to its normal size although the number of cells is decreased. Therefore the possession of a goiter does not necessarily mean an ever-present deficiency of thyroxine but indicates that there was a *deficiency at one time*.

Statistics of the number of goiters have been made in the United States by the Draft Board (figs. 32, 31) and certain school boards, and it is shown that there is more goiter in the northern part of the United States where there is less iodide than there is in the southern part where there is more iodide. In Utah there was less goiter among the school children in towns containing in the water more than 1 part of iodide per billion than there was in those containing less than 1 part of iodide per billion of drinking water. The few cases of goiter in Japan are in mountainous districts where no edible seaweed is obtainable.

In a goiterous region a certain proportion of the goiters become toxic (fig. 32). Toxic goiter is always less abundant than simple goiter. Both simple and toxic goiter are due to iodine deficiency and are benefited by taking inorganic iodide, but the dose required for toxic goiter is much higher than that for simple goiter; in fact, in treating simple goiter the amount required bears some relation to the deficiency of iodine in the body, allowing for the fact that there is a great waste of it in the urine.

In treating toxic goiter very much larger doses are required and even these must be increased, so that very few physicians are content with the results of such treatment and turn the patient over to the surgeon. Dautreband compares the treatment with iodine in potassium iodide (where the doses are increased sufficiently) to the treatment by surgery.

The toxic goiter is associated with, if not the direct cause of, a high basal metabolic rate, and the basal metabolic rate is the only quantitative index of the disease. In the table on p. 102 cases of about the same basal metabolic rate are chosen for comparison (Dautreband). High rate is due to muscle tone.

Bourcet and Gley discovered iodide in the blood in 1900. Kendall and Richardson found 130 parts per billion. Sturm claims that iodide is equally distributed between the corpuscles and the plasma, and found the average blood iodide to be 130 in

autumn and 80 in winter. Blum and Grützner (1913) precipitated the proteins with acetone and determined iodine in precipitate and filtrate. Veil and Sturm, 1925, attempted to fractionate the iodine compounds in blood by precipitating the proteins with alcohol and extracting the lipoids with chloroform. That remaining in the water and not precipitated with the protein was considered inorganic. Blum (and later Lunde) think that the blood proteins contain thyroglobulin. Sturm, 1928, claims that blood iodine is reduced after thyroidectomy, but Hudson, 1922, found it increased. DeQuervain and Smith, 1928, found 60 parts per billion in cretin blood and 130 in normal blood, and claim there is no rise in cretins after administering potassium iodide. Ishikawa found 11 parts per billion in normal blood and 26 in toxic goiter blood.

Prolonged Iodine Treatment			Short Iodine Treatment and Operation			
Previous B.M.R.	B.M.R. after treatment	Duration of treatment	Previous B.M.R.	B.M.R. before operation with I <sub>2</sub>	B.M.R. after operation with I <sub>2</sub>	B.M.R. after operation without I <sub>2</sub>
+80	+29	5 mo.	+80	+37	+32	+60
+67	+14	5.5 mo.	+67	+28	—	+30
+65	+31	2 mo.	+50	+17	+13	+13
+64	+11	1 yr.	+66	+4	+10	+4
+60	+25	2 mo.	+60	+30	+30	+55
+43	+6	14 mo.	+44	+10	-42	-23
+40	-1	6 mo.	+40	+10	+10	+50
+40	+10	2.5 yr.	+40	-10	-25	-15
+39	+16	2.5 yr.	+50	+8	-3	+0
+39	-6	3 mo.	+43	+12	-11	+31
Aver. 53.7	13.5		57.0			20.5

Blum, Romeis, and others claim that blood contains an anti-thyroxine. There has been much discussion of the lowered basal metabolic rate following the feeding of very heavy doses of iodine in potassium iodide to persons with toxic goiter. Uhlenhuth from studies on transparent tadpoles claims that iodine causes a retention of thyroglobulin in the thyroid and supposes that the high basal metabolic rate in toxic goiter is due to too much thyroglobulin being passed into the blood and that heavy doses of iodine cause it to be retained. The difficulty with this interpretation

is that no thyroglobulin has been found in toxic goiter tissue, which would indicate that it had to be thrown out immediately after its production, which is contrary to the findings in regard to any other secretions.

Marine showed that, on giving iodide to the hyperplastic gland, thyroglobulin (colloid) is produced (dried hyperplastic gland contains about  $0.01 + \%$  and colloid gland about  $0.1 + \%$ ), and Williamson showed that the hyperplastic gland getting a little iodine or iodide has thyroglobulin formed in only certain places and it is only these regions that will cause tadpoles to metamorphose. Preston, working under Kendall, showed that there is no thyroglobulin or thyroxine in the cells but that it is all in the colloid material.

Marine and Kimball showed that sodium iodide will prevent goiter in school children, and this method is used in Switzerland and parts of the United States. The Swiss do not rely on the quantities given by Marine but on some older observations in Switzerland. The interdigitating cantons of Freiburg and Vaud (Waadt) had government salt monopolies and goiter examinations by the Draft Board as well as analysis of the salt. The Freiburg salt contained no detectable iodide, and goiter was high. The Vaud canton owned a salt mine at Bex which contained an appreciable amount of iodide, and it had a low goiter rate. The Swiss cantons nowadays sell an iodized salt (Vollsalz) which contains as much iodide as some old analyses of the salt of Bex showed.

Iodides are non-toxic. The daily prophylactic dose is about 0.02 mg., whereas some syphilitics take 10,000 mg. in a day without toxic symptoms.

Iodide is doubled in the blood in nephritis. Lipschitz found that inorganic iodide is concentrated five times from the blood to the gastric or parotid secretion, but thyroxine is not secreted into the stomach.

The normal thyroid weighs 1 g. at birth and 15 g. in the adult. In some goiterous regions, 100% of the new-born had an enlarged thyroid which remained enlarged throughout life, although it was not large enough to be detected by palpation.

Owing to the fact that dried sponge and sponge-ash were used to cure goiter long before iodine was discovered, a great deal of emphasis has been placed on the iodide of the sea. There is more

iodide in the Great Salt Lake water than in sea water, but goiter is prevalent right up to its shores. The difference seems to be that people near the sea eat sea food and in that way get iodide, whereas no food for man grows in the Great Salt Lake.

Thyroid is relatively non-toxic to children, to exophthalmic goiter patients and to birds. Feeding birds huge doses makes the feathers grow more rapidly. The life of the feathers is shorter and molting occurs at more frequent intervals. Sometimes all of the feathers come out at the same time and the feathers may all come in with a different color.

Hektoen has determined thyroglobulin in thyroid vein blood by means of immune reactions (serology). It has been stated that there is more thyroid secretion in the blood in toxic goiter. It must be remembered, however, that toxic goiter, although of unknown origin, never develops in a patient that has not at one time had a deficiency of iodine. How do heavy doses of iodine in potassium iodide lower the basal metabolic rate? Lunde claims that they increase the inorganic iodide of the blood but not the iodine in the protein; in fact, he claims it decreases the latter. He thinks that the iodized protein in the blood is thyroglobulin. It should be remembered, however, that the action of elemental iodine on any protein produces diiodotyrosine in its molecule, and the feeding of such proteins does not raise the basal metabolism in man whereas the feeding of thyroglobulin does raise it. Thyroxine or thyroid has been known to lower the basal metabolic rate in exophthalmic goiter patients and should be tried as a remedy.

Dautrebande: *Physiopathologie de la Thyroïde*, Paris (1931).

Lunde: *Nordish. Med. Tidsskrift* 1:475 (1929).

McClendon: *Physiol. Rev.* 7:189 (1927).

Von Fellenberg: *Ergebnisse Physiol.* 25:176 (1926).

**Iodates** are used in yeast food. Sollmann claims that 0.3 g. per kg. given orally is harmless but the same dose administered hypodermically is fatal. Iodates are found in "caliche" (Chile saltpeter). The question of whether iodates exist in the sea has not been settled. Sea water is alkaline, and one would expect iodide to be oxidized to iodate by atmospheric oxygen. Sunlight and traces of heavy metals may act as catalysts. Winkler attempted to separate iodate from iodide in the analysis of sea

water. The enormous chloride content of the water makes this task difficult.

Sollmann: J. Pharmacol. 9:269 (1917).

Winkler: Z. angew. Chem. 29:205 (1916).

**Oxygen.** Paul Bert subjected small mammals to increased oxygen pressure and claimed that he obtained toxic effects. Whether this was due to impurities in the oxygen has not been absolutely determined, but it seems probable that oxygen under high pressure itself is toxic.

With small organisms the rate of oxidation in the tissues seems to depend on the oxygen tension in the water surrounding them. This may, however, be merely the rate of diffusion of oxygen to the point where it is utilized, in which case oxidation would be stopped by total lack of oxygen but not retarded by diminished concentration.

Certain organisms like the jellyfish, *Cassiopea*, may live for about 10 hours in the absence of oxygen, in which case no carbon dioxide is produced and paralysis of all life processes occurs. On admission of oxygen the animal awakens and starts to produce carbon dioxide.

Anoxemia of the tissues is supposed to occur in pneumonia, bronchitis, asthma, heart disease, and anesthesia. Anoxemia during anesthesia can be prevented by the addition of carbon dioxide to the gas mixture, thus increasing the rate of respiration by the action of the carbon dioxide on the respiratory center.

Anoxemia is a serious problem in mountain climbing and flying. Haldane claims that anoxemia produces progressive and sometimes irreparable damage to living tissue. Barcroft believes that severe anoxemia, as in mountain climbing, may take days or weeks for recovery but that complete recovery is impossible in elderly or unsound persons, and that anoxemia affects the brain and produces physical and mental fatigue. Anoxemia for about 5 days, as in attempts to climb Mt. Everest, results in irreparable damage. During anoxemia there is an increase in the heart-rate as well as respiration.

During a sojourn at low oxygen pressure there is an increase in the number of red blood cells, and this increase is supposed to be one of the benefits derived from a vacation in the mountains. MacLeod observed a rise in lactic acid in the blood following acute

anoxemia. Keefer believes that angina pectoris is due to anoxemia of the myocardium.

Riddle states that females survive decreased pressure of oxygen better than males, who have a higher basal metabolic rate. Such survival has been used in Europe as a test of basal metabolic rate in relation to the thyroid. Thyroid preparations are administered to small animals which are then kept at low oxygen pressure and compared with normal animals. If active thyroid hormone is present they die at higher oxygen pressures than are required to kill normal animals. On the contrary, if the thyroid is removed from these animals they live at lower oxygen pressures.

It is not possible to take in oxygen through the lungs and blood stream rapidly enough to supply the needs of violent muscular exercise, and so an oxygen-deficit is built up. This deficit is represented by the accumulation of lactic acid in the body, some of which may even pass out in the urine. After the exercise is over, rapid breathing occurs until the lactic acid is burned.

This same process, according to Gesell, also takes place in the respiratory center. The respiratory center is stimulated by hydrogen ions, irrespective of their origin, but these ions must be within the cells of the center itself in order to be effective. Normally carbon dioxide is the acid from which these ions are derived, and it is produced in the center itself. The carbon dioxide pressure of the blood retards its elimination, and so when the concentration of this substance reaches a certain value the respiratory center is stimulated. During anoxemia, lactic acid in the center stimulates it and breathing may be so violent that the carbon dioxide tension of the blood is greatly decreased. This occurs during mountain sickness. By this extreme breathing the blood may become more alkaline although some lactic acid enters it and chloride ions come out of the corpuscles and perhaps the muscle cells in exchange for  $\text{HCO}_3^-$  going in.

Barcroft: *Lancet* 2:485 (1920).

Haldane: *Brit. Med. J.* 2:65 (1919).

Keefer and Resnik: *Arch. Internal Med.* 41:769 (1928).

MacLeod: *Am. J. Physiol.* 55:175 (1921).

**Sulfur** exists in the body as inorganic sulfate, ethereal sulfate, ergothionine, cystine, chondroitin sulfuric acid, and mucoitin sulfuric acid. When muscle is built in the body, the retention nitrogen to sulfur ratio is about 14 (the ratio of nitrogen to sulfur

in muscle tissue). Since 88% of the sulfur in the urine is inorganic, during starvation when the body is burning its muscles for every gram of nitrogen excreted about 0.063 g. inorganic sulfur is excreted. With 100 g. protein a day on a diet of muscle this would be about 600 cc. 0.1  $N$   $H_2SO_4$  in the urine (1 equivalent of nitrogen weighs about the same as 1 equivalent of sulfur).

Ammonium sulfate acts as a diuretic, but this is partly due to urea formed from ammonia — though not entirely, since sodium sulfate injected intravenously acts as a diuretic. Diuresis produced by sulfate is apparently not related to its excretion, since it is not excreted as rapidly as chloride, and it is dammed up in the blood in nephritis.

Thiosulfate injected intravenously is useful in arsenic poisoning.

When sulfur is injected intravenously in colloidal form it is eliminated as  $H_2S$  in the breath, but colloidal sulfur is reported to have physiological activities differing from  $H_2S$ .

Sulfate in normal human blood serum varies from 0.4 to 1 milliequivalent per liter.

Both sodium and magnesium sulfates are poorly absorbed from the gut so that the action of epsom salts is not due to magnesium alone. The distension of the rectum causes defecation.

Thiocyanate varies from 0.03 to 0.06 mg. per 100 cc. in body fluids and is increased in tobacco users. A small amount of thiocyanate was detected formerly only in the saliva, but this is probably due to difficulty in analysis in fluids richer in protein. It may arise from cyanide or acetonitrile.

Ethereal sulfate arises by the conjugation of inorganic sulfate with phenol (or other poisons). The ethereal sulfate is less toxic than its precursor and is called a detoxication compound.

Some cystine is excreted in normal urine, larger amounts in the condition known as cystinuria.

Blum: *Z. klin. Med.* 107:61 (1928).

Denis, Herrmann and Reed: *Arch. Internal Med.* 41:385 (1928).

Lewis: *Physiol. Rev.* 4:394 (1924).

Möller: *Arch. exptl. Path. Pharmacol.* 126:143 (1927).

**Selenium** occurs in the soil, and toxicity to stock of grain and fodder grown on certain "alkali" soils in South Dakota is attributed to selenium. Gassmann found about 0.056% in teeth and 0.001–0.009% in urine. Muehlberger found the minimum lethal dose of intravenous colloidal selenium to be 6 mg. per kg. Hurd-

Karrer found that 1 part of selenium was detoxicated by 12 parts of sulfur added to the soil.

Gassmann: J. Chem. Soc. 110(1):772 (1916); 112(2):540 (1917).

Hurd-Karrer: Science, 78:560 (1933).

Muehlberger and Schrenk: J. Pharmacol. 33:270 (1928).

**Nitrates** are readily absorbed. Practically the entire quantity is excreted unchanged, but a small percentage may be reduced to nitrites. Large doses may cause methemoglobin formation (Cole). They are used in brightening the color of hemoglobin in corned beef, bacon, and ham.

Some western soils contain 1-2% nitrate, and some saline crusts in the Sahara Desert contain 6%. The chief deposit is in Chile and is known as "caliche." The artesian water of southern California contains nitrates and iodine, suggesting contact with deposits similar to caliche.

Nitrate is formed in thunderstorms and comes down in rain. It is formed by nitrogen-fixing bacteria in the roots of legumes. Nitrate is said to be an accelerator of zymase action and to cause contraction of the arteries. When more than 7 g.  $\text{KNO}_3$  is fed to the mother it leads to nitrate excretion in the milk.

Cole and Paryzek: J. Am. Med. Assoc. 68:1089 (1917).

Guillaume: Am. Mines 9:139 (1924).

Stearns: Am. Mineral. 9:135 (1924).

**Nitrites** lower blood pressure.

**Arsenic** (similar to antimony and bismuth) is a tri-pentavalent element and is used mainly in combating protistan diseases (non-bacterial). It has been claimed that it never kills all of the protista in the body but that some are killed and liberate antigen. Syphilis, malaria and similar diseases are treated in this way. Trivalent arsenic is more toxic than pentavalent. Inhabitants of Styria are said to take 2-3 g. a day.

Eight milligrams of arsenic are excreted in the urine of an adult per year, ordinarily (Klason). Gautier found arsenic in human tissues. Remington found arsenic in tobacco, probably due to spraying the leaves with an insecticide. Segale claims that the thyroid gland is the highest tissue in arsenic content, with 70 parts per million.

The minimum lethal dose of arsenic is 130-260 mg. In small doses it has been used since ancient times therapeutically. It is

excreted in the urine and hair. Metallic arsenic rubbed on the skin produces lesions (Muller). The nutrition of the skin and hair is greatly stimulated by optimal amounts, and arsenic is used by veterinarians in fitting animals for show purposes. Most arsenicals that are used in medicine are organic and liberate inorganic arsenic very slowly.

Gautier: Compt. rend. soc. biol. 54:727 (1899).

Muller: Arch. Dermatol. Syphilol. 15:186 (1927).

Segale: Z. physik. Chem. 42:175.

Underhill: J. Biol. Chem. 76:163 (1928).

**Antimony, Sb.** Thirty milligrams of tartar emetic causes vomiting; 1.74 g. is known to be fatal. Trivalent antimony is 10 times more toxic than pentavalent.

Henderson and Taylor: J. Pharmacol. 2:153 (1910).

**Bismuth** is used chiefly as the insoluble compounds, such as the subnitrate used in X-ray studies of the gut. Bismuth has a depressant action, and sufficient amounts of bismuth subnitrate are dissolved to show this action on the gut. Like other heavy metals, bismuth damages the kidney. It is excreted, however, through the intestine as well as in the urine and it has been found even in the saliva. Ordinarily about 65% is excreted in the urine and 35% in the feces. Like other heavy metals it causes diuresis due to damage to the convoluted tubules.

Hanzlik: J. Am. Med. Assoc. 92:1413 (1929).

Meyer and Steinfeld: Arch. exptl. Path. Pharmacol. 20:40 (1885).

**Silicon.** Six-tenths milligram of  $\text{SiO}_2$  is present in 1 g. of vitreous humor of the eye. It occurs in feathers, and to the extent of 2% in blood-ash. Diamond-mine workers and those working with hard stone may develop silicosis due to finely divided  $\text{SiO}_2$  in the lungs and are more susceptible to tuberculosis than ordinary persons.

A large number of compounds in which carbon is replaced by silicon have been synthesized, and Drechsel claims silicon to occur in organic compounds in feathers.

Metasilicate of soda is useful in cleaning glassware. Traces of it should be added to synthetic experimental diets.

Drechsel: Zentr. Phys. 11:361 (1918).

Heffernan and Green: J. Ind. Hyg. 10:272 (1928).

Schulz: Biochem. Z. 70:464 (1915).

**Germanium** is said to stimulate the formation of red blood cells. The lethal dose of  $\text{GeO}_2$  is 0.5 g. per kg. It occurs to the extent of 0.05% in some meteorites.

Goldschmidt and Peters: Nach. Gesell. Wiss. Göttingen (Math.-Phys.) 1933:141.

Hammett: J. Exptl. Med. 35:173 (1922).

**Tin**, Sn, is toxic in large doses of its soluble salts. It is used in weighted silk. Sensitivity to this silk is said to be due to liberated formic acid from the compound (formate) rather than to tin.

Metallic tin is used for food-containers because it coats over with a film of oxide which prevents further oxidation in the air. It dissolves, however, in certain food juices.

Tin salts have been used as a preservative for cider (1-2%). Huge doses produce symptoms similar to lead poisoning.

Tin is eliminated both by the kidney and the gut, chiefly by the latter.

Salant and Rieger: Proc. Soc. Exptl. Biol. Med. 11:178 (1914).

**Cerium** is a depressant and is said to act especially on the vomiting center. It is very toxic, a solution 1:10,000 inhibiting plants. With cerium poisoning there is a loss of hair.

Cerium acetate or sulfate dissolved in alcohol is used as a catalyst in ashing biological material. The dry material to be ashed is moistened with the solution and the alcohol evaporated.

Saburo: Arch. Exptl. Path. Pharmacol. 100:217 (1923).

**Vanadium** has been found in animals. In the ascidian *Phallusia* it is said to take the place of iron in a respiratory pigment.

Henze: Z. physiol. Chem. 72:494.

**Lead**, Pb, is greatly feared as a poison. Painter's colic is due to particles of lead carbonate breathed into the lungs, or absorption of lead by the skin.

Lead is stored in the bones as phosphate and under certain conditions comes out of the bones to give a second poisoning. Change in the acidity or basicity of the diet sometimes causes it to come out. Ammonium chloride and acids increase the excretion of lead, probably bringing it out of the bones. In chronic lead poisoning, 0.12-0.78 mg. is excreted in three days' urine, and it is probable that larger amounts go out in the feces. It is

stated that enormous doses of  $\text{Na}_2\text{CO}_3$  increase its excretion. This is probably due to the diuretic action of the carbonate.

Aub and collaborators: Med. Monographs, Baltimore (1926).

**Chromium** is used in chrome-plating and chrome-tanning. It takes less time to tan with chromium salts than to tan with tan bark. The lethal dose is 8 g. bichromate.

Brieger: Z. exptl. path. Therap. 21:393 (1920).

**Molybdic acid** is a germicide. Phosphomolybdic acid is used in precipitating alkaloids and proteins. In chemical analysis it is used for the determination of reducing substances.

Walburn: Acta Path. Microbiol. Scand. 3:489.

**Manganese** has some relation with the metabolism of iron. Manganese occurs in traces in all human organs. Blood contains 0.01 mg. per kg. of plasma, but none is found in the corpuscles. Urine contains less than 1 part in 50,000,000. It is excreted mainly by the intestine. Traces of it should be added to synthetic experimental diets. It is said to replace iron in respiratory pigment of mussels (pinnaglobin).

Manganese and iron are necessary for chlorophyll formation in plants. It is higher in leafy vegetables and lower in fruits.

A 0.1% solution of permanganate is antiseptic. Taken internally in large doses it causes death by gastroenteritis. It can be used to purify water and wash raw fruits and vegetables. It is used in oxidation-reduction titrations. It is reduced by sunlight and dust.

Bertrand and Sazerac: Bull. chim. 15:627 (1913).

Bradley and Morse: J. Biol. Chem. 21:209 (1915).

Reiman and Minot: J. Biol. Chem. 42:329 (1920).

**Iron, Fe, and copper, Cu,** are concerned mainly but not entirely with hemoglobin and cytochrome. Barkam and Berger found hemoglobin to contain 65% of the iron of the blood. There is iron in cells containing no hemoglobin and very little cytochrome. The iron absorbed from food is never in the form of hemoglobin. In growing animals the relative iron content has been estimated by the hemoglobin content of the body.

Since the hemoglobin is used to carry oxygen, and oxygen is used to produce heat, the hemoglobin content of the body has

some relation with the average metabolic rate over long periods and perhaps with the basal metabolic rate. Since the basal metabolic rate varies with surface area and with age and sex, the hemoglobin in the body varies in the same way except during iron-starvation or anemia from other causes.

Ordinarily there is a superabundance of iron in the food. The only natural food deficient in iron is milk. An infant ordinarily nurses exclusively for about ten months. In some families the infant may obtain the majority of its calories from milk for several years. The Shinto priests in Japan nurse for about seven years.

There has been considerable argument over the question of whether infants have an iron deficiency or whether they are born with an excess store of iron. It depends somewhat on the point of view. Thus, if we consider the iron per unit of surface area, the infant is not born with an excess of iron.

If one examines embryos, he is impressed by the large cross-section of the blood vessels. Georges Dreyer claims that the cross-sections of the blood vessels and the trachea are proportional to the body surface. In a study of white rats as shown in the following table the hemoglobin seems to be more nearly proportional to the two-thirds power of the weight. The hemoglobin is certainly not proportional to the weight.

HEMOGLOBIN IN RATS

Age in days	$(W)^{2/3}$	Hemoglobin	$Hb/(W)^{2/3}$	$Hb/W$
1.....	1.59 g.	0.026 g.	0.0164	0.013
6.....	2.83 g.	0.048 g.	0.0126	0.006
11.....	5.45 g.	0.064 g.	0.0117	0.005
22.....	8.00 g.	0.105 g.	0.0131	0.0046
28.....	10.30 g.	0.221 g.	0.0214	0.007
32.....	11.70 g.	0.296 g.	0.0253	0.007

Bunge, who compared the hemoglobin to the weight, supposed that mammals are born with a store of iron. That led him to look for iron in the eggs of birds, and he found an iron compound which he called hematogen. In the above table, there is only a very slight *decrease* in the hemoglobin per unit surface down to the twenty-second day, which is the end of the nursing period, and then a greater increase in the hemoglobin after the rat eats a mixed diet. According to surface area the rat is *born with a*

*slight deficiency* of hemoglobin and the deficiency gets worse through the nursing period.

The surface area of humans is about 0.18 sq. m. at birth, 0.44 at one year, 1.73 at seven years, and 1.86 for the adult. The blood volume is about 3,250 cc. per sq. m. of body surface, which would mean (by calculation) 585 cc. at birth, 1,430 at one year, 2,370 at seven years, and 6,450 for the adult. The hemoglobin content of the blood (Williamson, fig. 33) at birth is 23.25%, at one year 12.45%, at seven years 14.37%, and for the adult about

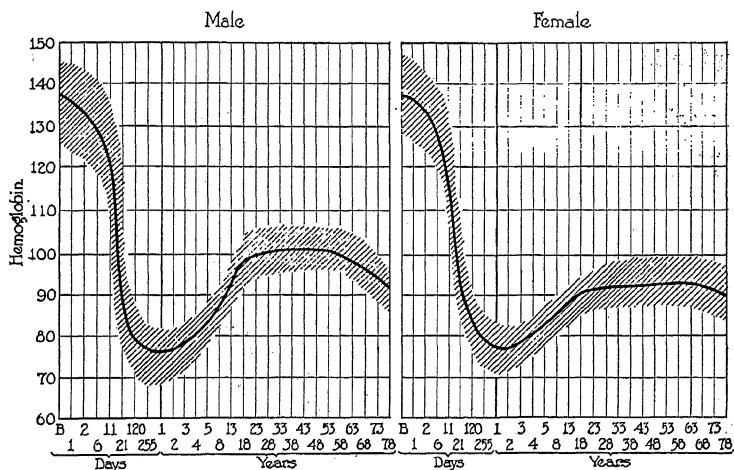


FIG. 33. Williamson's curve of hemoglobin values with age and sex. The shaded area represents approximately the standard deviation.

16% (average for the two sexes). Therefore the total hemoglobin in the body would be 135 g. at birth, 178 at one year, 340 at seven years, and 1,032 for the adult. The hemoglobin per square meter is calculated at birth 756 g., at one year 405, and for an adult 520, but these figures are no more accurate than those of blood volume. Williamson's data (fig. 33) would not indicate so great a hemoglobin storage at birth in humans.

Chlorosis is an anemic condition chiefly in adolescent girls, the cause of which is not very clearly understood and which is disappearing. Since it was shown that, with change of clothing, chlorosis became less common, it was thought that ultra-violet light had something to do with hemoglobin formation, but this

has never been proved. The sex difference may be due to the loss during the menstrual flow.

Williamson concluded that inorganic iron, whether given by mouth, subcutaneously, or intravenously, is deposited in the liver and spleen, not in the form of hemoglobin. Whipple studied the blood-regenerating ability of various foods and found red meat and cooked liver the best. Hemoglobin and butterfat come next, spinach third, and cereal grains and milk last.

Iron is eliminated partly in the urine and partly by way of the intestine.

It would seem that there are few cases of iron-deficiency, except in infants, and that the other constituents of hemoglobin are the ones that limit its production, but even in the nursling the hemoglobin production seems not to depend entirely on iron. Waddell, Steenbock, and Hart found that ferric chloride would not serve to cure milk-anemia but copper is also necessary (there is 0.5 mg. copper per kg. milk).

#### RELATIVE IRON AND COPPER CONTENT OF FRESH VEGETABLES

<i>Iron</i>		<i>Copper</i>	
Lettuce.....	100	Carrot leaves.....	100
Carrot leaves.....	65	Sweet potatoes.....	86
Spinach.....	64	Potatoes.....	67
Beet leaves.....	26	Beet leaves.....	60
Turnip leaves.....	22	Onions.....	60
Sweet potatoes.....	22	Okra.....	56
Carrots.....	15	Carrots.....	50
Potatoes.....	14	Turnip leaves.....	48
Beets.....	14	Asparagus.....	47
Onions.....	13	Spinach.....	45
Beans, green.....	12	Beets.....	42
Asparagus.....	10	Beans, green.....	38
Turnips.....	10	Tomatoes.....	38
Okra.....	9	Squash.....	37
Cabbage.....	7	Lettuce.....	33
Tomatoes.....	5	Cabbage.....	23
Squash.....	5	Turnips.....	19

(From the laboratory of the South Carolina Food Research Commission.)

Of the foods high in iron, liver contains about 8 mg. per 100 g., wheat germ about the same, egg yolk almost as much, and spinach about 7; and of the foods low in iron, white flour contains 0.9 and milk 0.02. Lettuce and spinach are high in iron and cabbage

and tomatoes relatively low. Potatoes, sweet potatoes, onions, and the leaves of carrots and beets are high in copper.

PARTS PER MILLION, DRY BASIS, SOUTH CAROLINA  
VEGETABLES (REMINGTON)

	Ca	P	Fe	Mn	Cu	I
Beet tops.....	13660	5583	372	183	13.5	.546
Beet roots.....	2755	5803	141	76	9.1	.179
Carrot tops....	16963	4258	517	121	12.4	.404
Carrot roots...	6054	5052	204	42	10.7	.235
Turnip tops....	24500	4680	400	108	7.8	.376
Turnip roots...	6180	4660	123	14	4.4	.298

RELATION OF DIET TO HEMOGLOBIN IN SOUTH CAROLINA  
STUDENT NURSES (REMINGTON)

	Ten showing highest hemoglobin	Ten showing lowest hemoglobin
Fatty foods.....	75	75
Vegetables.....	247	141
Hemoglobin.....	14.3	10.6

In the vegetables at the Wisconsin Agricultural Experiment Station there were 5 times as much manganese and 15 times as much iron as there was copper. It was found that the addition of not only liver but also liver-ash to milk increased the hemoglobin in growing rats. If the copper was removed from the liver-ash, there was less rapid increase in hemoglobin.

Copper salts are sometimes used to give a bright green coloration to pickles, which may then contain up to 100 mg. copper per kg. The use of copper cooking utensils increases the copper in food but this causes more rapid destruction of vitamins.

Copper is a constituent of hemocyanin, which functions in certain invertebrates (molluscs and arthropods) just as hemoglobin does in vertebrates. Oysters are very high in copper and marine fish contain it, which has been shown by Vickery, Bodansky, and Remington. Sea water is said to contain 1 part in 5 million. Copper occurs in pigment gall-stones. Copper-bilirubin shows an absorption band at 6,512 Å and if present could be detected.

Sodium cupric tartrate is more easily absorbed by the gut than

inorganic copper compounds, but copper soaps are still more easily absorbed.

Mallory attributes hemochromatosis of the liver to copper in "moonshine" whiskey.

Copper sulfate is used in phosphorus poisoning because it coats the globules with copper and has an emetic action.

$\text{Cu}^{++}$  has a relatively high toxicity for fungi, protozoa, and algae, and has been used to kill algae in reservoirs. Most of it is precipitated, and the remainder is much less toxic to humans than it is to algae.

Workers in copper mines have been in apparently good health with green copper carbonate coating their teeth. Copper alloys have been used by dentists in the mouth, and the teeth may become coated with green (probably copper carbonate) without apparent detriment to health. The lethal dose of subcutaneous  $\text{CuSO}_4$  is 10 mg. for a rabbit and 80 mg. for a dog.

Elvehjem and Hart: *J. Biol. Chem.* 82:473 (1929).

Hart, Steenbock, Elvehjem, and Waddell: *J. Biol. Chem.* 65:67 (1925); 72:299 (1927); 77:777, 797 (1928); 83:243, 251; 84:115 (1929).

Robschey-Robbins: *Physiol. Rev.* 9:666 (1929).

**Cobalt** is a normal constituent of plants and animals, and Bertrand found it in insulin. It is not very toxic. Waltner, who added 0.5% powdered cobalt to the diets of rats, claimed that the erythrocytes were increased to over ten million per cubic millimeter and the hemoglobin to 165% of the normal.

Bertrand and Nakamura: *Compt. rend.* 185:321 (1927).

**Nickel** is a constituent of living things, and Bertrand found it in insulin. It may be present in hydrogenated fats, owing to its use as a catalyst. Bertrand and Nakamura attempted to cause a nickel and cobalt deficiency in young mice and found that controls lived longer on the addition of small amounts of nickel and cobalt. A nickel-rash occurring in nickel-platers is said to be due to exposure to excess heat and humidity, but Richter found nickel in the urine. Nickel carbonyl is toxic when inhaled.

Richter and Fairhall: *J. Am. Med. Assoc.* 49:1606 (1907).

**Zinc** seems to be essential for the normal growth of plants, and traces of it should be added to synthetic experimental diets since it occurs in animals. Large doses have an emetic action.

Zinc stearate used in dusting powder for infants sometimes causes chronic lesions in the respiratory and digestive tracts due to swallowing or insufflation. Zinc is excreted chiefly in the intestine as a sulfide.

Zinc salts are not very powerful antiseptics.

Bertrand and Vladesco: *Bull. chim.* 31:268 (1922); 33:341 (1923); *Compt. rend.* 173:176 (1921).

Schwartz and Alsberg: *J. Pharmacol.* 21:22 (1922).

Silver has been detected in marine animals.

Silver poisoning is characterized by nephritis as are other heavy-metal poisonings. Metallic silver is used in surgery, being allowed to remain for long periods of time attached to the bones. Silver-foil has been used over granulation of the tissues to prevent exuberance. Part of its action is keeping out ultra-violet rays, which are said to favor these granulations.

Silver nitrate is dropped in the eyes of infants at birth, being required by law in many states. The colloidal silver preparations have doubtful antiseptic values. The bactericidal power of colloidal silver is due to particles between 5 and 15  $m\mu$  in diameter.

$Ag^+$  inhibits the action of enzymes. Many enzymes recover their activity on the addition of  $H_2S$  or  $HCN$ .

Euler and Myrback: *Z. physiol. Chem.* 121:177 (1922).

**Cadmium** is five times as toxic as zinc. In cats the intravenous lethal dose is 2-3 mg. of cadmium. Twenty-five milligrams by mouth produces vomiting, diarrhea, and ulceration. Burdach swallowed 0.5 g. cadmium sulfate, which produced vomiting.

Four per cent cadmium sulfate was used as a disinfectant during the World War.

Salant: *J. Pharmacol.* 15:217 (1920).

**Palladium** has been injected to produce local loss of fat-tissue. Two cubic centimeters containing 80 mg.  $Pd(OH)_2$  are injected once a week into the abdominal fat.

Palladium is used to plate hydrogen electrodes as it absorbs hydrogen more rapidly than platinum and can be removed with  $HNO_3$ . It cannot be used in solutions of  $HCl$ ,  $H_2SO_4$ , or  $HNO_3$ , which dissolve it.

Gerber: *Compt. rend. soc. biol.* 68:938; 69:102; 70:551.

**Gold** occurs in animals. Large doses of its soluble salts by mouth cause vomiting. Its use in medicine ("Keeley cure")

is probably psychic although the salt of any heavy metal taken into the mouth paralyzes the sense of taste for some time and might in this way inhibit the desire to smoke or drink. Sea water contains 12 parts per billion.

Berg: *Biochem. Z.* 198:424 (1928).

**Mercury** in the form of its soluble salts is a widely used antiseptic and antisyphilitic as well as a cathartic and diuretic. The organic compounds liberate mercury ions slowly and are therefore safer. Sollmann showed that the diuretic action of organic mercury compounds, such as novasurol, is due to the liberation of mercury ions which temporarily suppress the action of the loop of Henle in re-absorbing the glomerular fluid. About 20 liters of glomerular fluid is filtered out of the blood per day, and if none were absorbed, there would be this volume of urine.

Calomel is used as a cathartic and has the additional action of being an intestinal antiseptic. Violent gastroenteritis follows too large a dose. The cathartic action of mercury is controlled partly by the use of poorly soluble compounds, such as calomel or mercurous iodide.

The addition of an oxidizing agent, such as iodine, may cause poisoning even in the skin that has been exposed to mercurous salts. Mercurous nitrate is used in purifying mercury of baser metals in the laboratory, and it penetrates into the skin of the hands. If they are stained with iodine, sloughing of the skin may occur.

Stock has promoted a great fear of mercury. He has shown that the use of metallic mercury by laboratory workers results in detectable quantities in the urine, and he found cases of acute mercury poisoning in such workers who spilled mercury in constant-temperature rooms with no ventilation. The symptoms he attributed to chronic mercury poisoning such as loss of memory, were, however, not quantitatively measured.

Animals are poisoned in respiration apparatuses in which air passes over mercury.

Cole, Rauschkolb, Schreiber, and Sollmann: *Arch. Dermatol. Syphilol.* 20:176 (1929).

Stock: *Med. Klinik.* 22:1250 (1926).

**Thallium** is used to poison rodents. It causes loss of hair and has been used in depilatories (Sabourand), but the lethal dose is

close to the depilatory dose and when rubbed on locally, on the beard for instance, it is absorbed and may cause loss of hair on the top of the head. Ten milligrams per kilogram in adults caused violent gastroenteritis; 8-9 mg. per kg. per day was shown to be toxic in children up to twelve years of age.

Davis and Andrews: Deut. med. Wochschr. 55:1546 (1929).

**Iridium** is useful in coating hydrogen electrodes as it absorbs hydrogen much more rapidly than platinum.

Gros and O'Connor: Arch. exptl. Path. Pharmacol. 64:456 (1911).

**Osmic acid** is injected to kill nerves. The therapeutic dose is 0.2 cc. of 1% solution.

Kosakae: J. Path. Bact. 23:425 (1920).

Segre: J. Am. Med. Assoc. 62:819 (1914).

**Tungsten** is useful chiefly as tungstic and phosphotungstic acid for precipitating proteins and alkaloids. The lethal dose of sodium tungstate by mouth is 0.55 g. per kg. and hypodermically 0.45 g. per kg. body weight.

Karautassis: Bull. Sci. Pharmacol. 31:561 (1924).

**Tantalum** is used in making surgical instruments which are resistant to acids or red heat.

Darwillier: Compt. rend. 183:193 (1926).

**Radium** is very toxic and is excreted mainly through the feces but partly by the kidneys, and when deposited in the bones, still exerts its toxic action by radiation.

Zwaardemaker claims that it may be substituted for potassium in Ringer's solution when used in an amount that would produce the same radioactivity. The radioactivity of sea water corresponds to  $1.4 \times 10^{-12}$  radium.

Radium is used chiefly to inhibit or kill tissues such as cancer or benign tumors. This is possible owing to the fact that cancer tissue is less resistant to the rays than many other tissues.

Stenstrom and Lohmann studied the action of such radiation on the decomposition of tyrosine in solution and found that the action was linearly proportional to the dosage.

Field: Am. Med. 31:435 (1925).

Fricke and Morse: Am. J. Roentgenol. Radium Therapy 18:426 (1927).

Stenstrom and Lohmann: J. Biol. Chem. 79:673.

Zwaardemaker: Veslag Wetenschappen Akad. Amsterdam, 25:517 (1916).

**Platinum** has a physiological action similar to other heavy metals (Gelpke). It is useful in fusing through glass in making electrodes, and in ashing biological material. When the latter contains much phosphorus it may ruin the platinum by alloying with it.

Gelpke: Arch exptl. Path. Pharmacol. 89:280 (1921).

**Uranium** is the heaviest element, its atomic weight being 238.03. A subcutaneous injection of uranyl nitrate of 3 mg. per kg. body weight is fatal in 5 or 6 days and 2 mg. per kg. body weight of dog is fatal in 10 days. It is used in the production of experimental nephritis. Rabbits injected with increasing amounts can stand 50–100 times the initial lethal dose without injury.

MacNider: J. Exptl. Med. 49:387 (1929).

**Radiation** is characteristic of Ra and U; it is a form of energy which can be propagated through space without the need of any material medium in which to travel and without the motion of any material substance to carry it. Radiation is detected by its reactions, which may be chemical, thermal or electrical in nature, with the substance on which the radiation falls after its passage through space. It is a form of energy of special importance for life. This is evident when we consider that light, a kind of radiation, is necessary for the formation of organic compounds in plants. Without these compounds organic life could not exist. Huygens in 1678 explained the transmission of radiant energy as a wave motion in space. Fresnel made a further addition to this theory of the mechanism of energy propagation in 1814 by assuming that the radiation traveled as a transverse wave motion through a non-material, perfectly elastic medium called the "Ether." The wave motion due to the transmission of radiant energy through this "ether" causes a displacement of the ether at right angles to the direction in which the energy travels, as a floating object on the surface of a lake is moved up and down as a wave travels over the surface. This is somewhat different from the wave motion due to sound. As a sound travels, the air molecules are displaced backwards and forwards in the same direction in which the wave is propagated. The recent experiments of Michelson and Morley, however, have thrown grave doubts on the existence of an ether while the relativity theory does not require such a concept. In the electromagnetic theory of light wave motion is no longer con-

sidered mechanical but is electromagnetic in character. Radiant energy consists of indivisible units known as quanta just as electric charges are in indivisible units known as electrons. In homogeneous radiation a certain number of electromagnetic vibrations are produced per second. For each vibration the energy is transferred a certain distance called the wavelength. The velocity of the radiation is therefore equal to the number of vibrations per second (the frequency) multiplied by the wavelength. All types of electromagnetic radiation have the same velocity, namely 299,860,000 meters per second. The wavelength ( $\lambda$ ) can therefore be used instead of the frequency  $f$  to characterize the type of radiation since  $\lambda = \frac{C}{f}$ . The wavelength of radiation can be studied and determined by means of a spectrograph. A homogeneous radiation is often referred to as a line because it shows up as a line in the spectrograph. The green line from a mercury arc, which is used to a great extent for polariscopes, has a wavelength of 5461 Ångström units (Å). Wavelengths are measured in either Ångström units or millimicrons ( $m\mu$ ) which are connected by the following relation:

1 millimeter = 1000 microns ( $\mu$ ) = 1,000,000  $m\mu$  = 10,000,000 Å.  
Radiation energy is transmitted through space by waves of a large variety of wavelengths, each group of wavelengths having characteristic properties. The whole electromagnetic spectrum may be divided into the following classes:

**Radio Waves.** Radio waves are more or less familiar to everyone as being the electromagnetic waves travelling through space carrying the radio programs. The wavelengths of the waves used in radio broadcasting extend from 1 to a 1000 m. Radiation of this wavelength at the present time is of little interest to the medical practitioner or the chemist. An interesting point in this connection, however, is the fact that the high frequency current used for heat treatments in physical therapy, has a frequency range which is the same as that of the radio stations. In diathermy an electrical current traverses the tissue, the human body producing heat in the tissues due to their electrical resistance. The current is made to alternate very rapidly, from 500,000 to 3,000,000 times a second, in order that no electrical shock is felt by the tissues. This rapidly alternating current acts as a source of electromagnetic radiation of wavelengths in the radio broadcasting range, a fact

which is fully appreciated by anyone having a radio receiving set close to a diathermy machine.

**The Hertzian Region.** Radiation of a wavelength from 0.01 to 100 cm. has been little studied and is of no known importance to the physiological chemist. It is of historical interest as the link between light radiation and the electromagnetic radiation from an electrical circuit through which an alternating current is flowing.

**Infra-red Radiation.** The solar energy as it strikes the earth is converted into heat maintaining this planet at a temperature suitable for living organisms. The greater part of the solar energy converted into heat lies in the infra-red section of the solar spectrum (7000–30,000 Å). Use is made clinically of the heat producing rays of the infra-red radiation from carbon arcs and electric light bulbs of high power consumption. Direct and reflected radiation, rich in infra-red rays, is directed onto the surface of the body and there converted into heat.

**Visible Light.** Radiation of a wavelength from 4000 to 7000 Å is capable of stimulating the retina of the eye and producing the sensation of light, hence the term "visible" as applied to this region of the electromagnetic spectrum. The maximum sensitivity of the eye is at a point of the solar spectrum which has the greatest intensity, this point being at about 5500 Å. These rays may produce chemical effects.

**The Ultra-violet Region.** The visible spectrum extending from a wavelength of 4000 to 7000 Å fades away at the lower wavelengths into a deep violet color. The region from 1000 Å to 4000 Å is known as the ultra-violet and it is with radiation from this region that many important photochemical reactions occur. A rather small section of the invisible ultra-violet part of the solar spectrum is responsible for the erythema, tanning and antirachitic effect of sunlight.

**Far ultra-violet and soft roentgen rays.** This region includes wavelengths of from 1000 Å to 1 Å. Due to experimental difficulties this region has been investigated only to a slight extent and to our present knowledge is of no physiological importance.

**Hard roentgen rays.** The wavelengths in this region extend from 0.06 to 1 Å, and radiations of this region are emitted when high speed electrons impinge on the surfaces of heavy metals. Such radiations have considerable chemical effect and are clinically

important in roentgen ray photography for diagnosis and for the treatment of cancer.

**Gamma rays of radium.** Radioactive materials on disintegration emit a radiation of very short wavelengths, the wavelength region extending from 0.001 to 0.6 Å. These radiations are clinically important in the treatment of tumors.

**Cosmic radiation.** The radiation of the shortest known wavelength are the cosmic rays, believed to originate from some extra-terrestrial source. These rays are extremely penetrating and are quite a recent discovery, so recent in fact that if they do possess any physiological significance, it is as yet unknown.

The most important radiation is that from the sun which supplies the earth with light and heat. This radiation consists of all wavelengths from far out in the infra-red to about 3000 Å in the ultra-violet. It is most intense at a wavelength in the green region of the visible spectrum. The solar spectrum is crossed by numerous dark lines known as Fraunhofer lines, believed to be due to absorption of the light of wavelengths corresponding to those lines of elements in a gaseous atmosphere surrounding the sun. An ordinary electric light bulb or a flame would give a continuous spectrum extending from the far infra-red through the visible and the upper portion of the ultra-violet. However, the greatest intensity would be in the red or infra-red region and only a small portion of the radiation energy would have wavelengths lying in the visible and ultra-violet. The chief source of ultra-violet for medicinal purposes is sunlight. The source of artificial ultra-violet radiation in most common use at present is the mercury lamp. The radiation does not extend over a continuous range of wavelengths but is grouped into several homogeneous types, which appear as lines or bands when dispersed by a prism or grating. Such line spectra are also produced by glow discharge tubes and arcs.

Roentgen rays are produced by the impinging of electrons onto metallic targets in highly evacuated bulbs. A strong electrical field imparts to electrons released from a hot cathode a great velocity. As these electrons strike the metal, radiation takes place, the wavelength and also the penetrating ability of the radiation increasing with the velocity of the impinging electrons. Radioactive elements when disintegrating also emit a radiation, called gamma rays, of a wavelength equal to that of the most penetrating or "hard" roentgen.

Bodenstein divides photochemical reactions into two classes. In the first class are placed those reactions which follow the Einstein law: that is, one activated molecule is formed for every quantum of radiation energy absorbed. In the second class are the chain reactions where an absorbed quantum of energy may result in the production of several reacting molecules. Bodenstein pictures the latter reaction by the formation of an unstable intermediate product which when changing to the stable final end-product emits enough energy to form another intermediate unstable molecule. The process then continues until stopped by substances known as inhibitors.

In physiological chemistry, however, the reactants in a photochemical process are so very complex that it is difficult to determine the exact chemical mechanism. With such substances as chlorophyll and ergosterol, whose chemical formulae themselves are disputed, the physiological chemist must for the present be satisfied with a study of the photochemical products or the biological effects without explaining how they take place.

One of the most interesting photochemical contributions of recent years is that made by Baly and his colleagues, in connection with the photosynthesis of plant products. It had for a long time been suspected that plants, exposed to sunlight, converted carbon dioxide into an active formaldehyde with the release of oxygen. Baly, using ultra-violet light of a wavelength around 2000 Å, was able to produce formaldehyde from carbon dioxide and water. With a slightly longer wavelength, 2900 Å, he was able to produce reducing sugars from formaldehyde. The presence of colloidal particles such as ferric hydroxide, malachite green and others, increased the concentration of reducing sugars of a solution in which carbon dioxide, formaldehyde and reducing sugars were in equilibrium. The presence of the colloidal particles also enabled the formation of reducing sugars to occur with visible radiation. This work was further extended by Baly and his colleagues to include the photosynthesis *in vitro* of nitrogenous plant compounds. Naturally occurring amino acids and alkaloids were produced from the reactions of potassium nitrate and ammonia with formaldehyde.

The curative effect of sunlight and ultra-violet radiation in rickets has been assumed to be due to photochemical formation of vitamin D from some sterol, probably ergosterol, in the skin.

Bills, Honeywell, and Cox have shown that unirradiated ergosterol has no antirachitic potency. Irradiation of ergosterol produces some activated product which can prevent rickets. Excessive radiation of ergosterol reduces the antirachitic potency. Ergosterol shows characteristic absorption bands in that spectral region which Sonne and others have shown to possess antirachitic properties. On irradiation these absorption bands disappear. Marshall has shown that the region responsible for this activation lies between 2800 and 3100 Å.

Proteins when exposed to ultra-violet light are coagulated in a manner similar to the coagulation which occurs on heating. This property of light coagulation has been studied by Adolf, who has suggested that the varied reactions of protein to ultra-violet radiation could be used as one of their properties for identification. Sonne has shown that the spectral region which is responsible for the coagulation of protein lies between the wavelengths 2270 and 3130 Å.

Bacteria irradiated in a medium which is transparent to ultra-violet rays are killed if the intensity and the time of exposure is great enough. Sonne has shown that the bactericidal region of the spectrum extends from 2270 to 3130 Å. In blood serum Eidinow has shown that tubercle bacilli are protected against ultra-violet rays. He attributes this to the protective layer of protein surrounding each bacillus, since it is known from the spectral absorption measurements of Lewis that proteins absorb strongly the bactericidal rays of the ultra-violet spectrum. Ultra-violet radiation does possess a bactericidal effect in vivo, since Colebrook, Eidinow and Hill have shown that the blood of rabbits after irradiation acquired the ability to kill staphylococci. The bactericidal action of ultra-violet light, however, is believed to be indirect when the animal body is irradiated, due to the small penetration of ultra-violet through the skin and the shielding effect of the protective protein colloids. According to Jansen, some chemical substance must be formed in the irradiated skin which is toxic directly or indirectly to the bacteria affected.

The effect of ultra-violet radiation on the blood corpuscles has been studied by many workers with many conflicting results. Hardy attributes these various results to the fact that sufficient consideration has not been given to the radiation dosage which depends on the intensity and duration of treatment. Campbell

and Hill have noted a stasis in the vessels of the frog's mesentery due to the formation of thrombi of lymphocytes. When the ultra-violet rays were allowed to pass first through egg albumen no stasis was observed. Egg albumen and other proteins are opaque to radiations of from 2500 to 3100 Å. It is this region, then, which is responsible for the stasis of the blood vessels and also for bactericidal effects and protein coagulation. From a review of the literature Hardy concludes that a normal erythrocyte count may be increased or a low count raised to normal by ultra-violet radiation. On irradiation there is an increase in blood platelets. The lymphocytes of a rabbit decrease on irradiation but rise to normal after the treatment. According to Hardy, the rapidity of the rise to the normal count depends on the dosage and the rise is slower the greater the dosage. This decrease in the lymphocytes does not depend on the wavelength of the radiation in the ultra-violet but on the intensity. Ultra-violet radiation of 3000 Å increases the poly-morphonuclear leucocytes of the rabbit, according to Hardy.

Ultra-violet light, roentgen rays and the rays from radium are able to render enzymes inactive. Hussey and Thompson have studied the effects of radioactive radiations on trypsin, pepsin, and invertase. The chemical effect was attributed mainly to the  $\beta$ -radiation from radium emanation, which is not electromagnetic in character but consists of a stream of electrons emitted by the radioactive source. They found that the enzymes were inactivated according to the monomolecular law, that is, the logarithm of the activity of the enzyme decreased in proportion to the dosage. Using ultra-violet radiation, Hussey and Thompson found that the decrease in activity with dosage followed the same monomolecular law. Clark and Northrop have irradiated solutions of trypsin with roentgen rays. They have shown that roentgen rays inactivate the free, active trypsin which is in equilibrium with combined, inactive trypsin, according to the mass action law. Consequently, irradiation of concentrated solutions of trypsin has no effect, since free, active trypsin is formed from the combined trypsin as fast as it is destroyed by roentgen rays. With dilute solutions a decrease in activity occurs since a larger fraction is in the free form which is inactivated by roentgen rays. Rothstein has used different wavelengths, 0.72 — 0.23 Å, to inactivate trypsin. For the different wavelengths used no variation in the ability to inactivate trypsin was observed. Fricke and Morse have observed

that roentgen rays reduce ferric sulfate to ferrous sulfate, the amount reduced being directly proportional to the dosage.

The study of the biochemical effects of radiation to within the last few years has been to a great extent of a qualitative nature. As already mentioned the two important factors in radiation research are wavelength and radiation-intensity. This latter factor has been almost entirely neglected by most workers. The wavelength of the radiations have been quite well established but the physiological and chemical effects per unit of radiant energy absorbed for a given wavelength present an attractive field of research for future investigators in radiation. Many difficulties are encountered in trying to measure dosage. The established standard for roentgen rays is the ionization chamber in which the number of ions resulting from the reaction between the radiation and the gas molecules of the chamber are measured by the electric current which they conduct under the influence of an electrical field. This method requires sensitive apparatus and an experienced operator and is inconvenient. Since roentgen rays produce chemical effects in proportion to the dosage, investigators have sought for some suitable chemical compound which will react on radiation and with which the amount changed will indicate the dosage. The most suitable at present seems to be either the ferrous sulfate solution, used by Fricke, or tyrosine, as used by Stenstrom, in which the change is directly proportional to the dosage.

A similar problem exists in measuring the dosage of ultra-violet radiations. Chemical changes proportional to the ultra-violet dosage have been proposed as a measure of dosage. Hill has used the fading of a solution of methylene blue in acetone to study the ultra-violet radiation from the sky. He has shown that ultra-violet radiation which produces an erythema is responsible for the fading of the methylene blue and that the color change is proportional to the coloration of the skin erythema. Such a reaction is influenced by minute impurities. Clark has shown that ultra-violet radiation decreases the ability of a paint known as "lithopone" to reflect visible light. The decrease in the reflection factor, to a 50% limit, is directly proportional to the ultra-violet dosage.

**Radiation in Therapeutics.** For many years rickets flourished in crowded cities where congested living conditions, smoke and fog permitted only a small amount of sunlight to reach the inhabitants. Huldshinsky emphasized the necessity of radiation in 1919 and

used the mercury lamp for treatment of rickets, and produced at that time remarkable cures as demonstrated by X-ray photographs. Experimental rickets were produced in rats by a low phosphorous diet in the dark and were cured by exposure to sunlight. Hess showed that the inorganic phosphorus in the blood of rachitic rats was increased to normal values on exposure to sunlight. Steenbock showed that osteoporosis in cattle is prevented by irradiated hay. The rôle of sunlight in the treatment of rickets seems to be a formation of viosterol, vitamin D, from sterols present in the skin as Hess and Weinstock have shown that irradiated skin would prevent rickets when fed to rats, while non-irradiated skin did not possess this property. Sonne and Reckling, Hess and Anderson, and others have shown that the radiation which renders an animal antirachitic is the ultra-violet of a wavelength less than 3130 Å. Dorno and others have shown that ultra-violet radiation of the sun contains only a small part of this antirachitic radiation. The shortest wavelength of the sun's spectrum lies between 2900 and 3000 Å, and depends on seasonal and daily variations. Hence, there is only the extreme ultra-violet region between 2900 to 3130 Å which is antirachitic. Certain artificial light sources of ultra-violet, such as the mercury lamp or carbon arc light, emit radiations extending from the visible spectrum to a wavelength of 2000 Å and are rich in the rays of the antirachitic region. These lamps are now used extensively in clinical practice as a source of ultra-violet for their antirachitic and other therapeutic effects, and supply the beneficent ultra-violet rays in places where the ultra-violet radiation of the sunlight is deficient. These rays penetrate water to a considerable distance.

A few hours after over-exposure to summer sunlight an erythema appears, the severity of which depends, to a considerable extent, on the thickness of epidermis; some blondes have a thick epidermis and are relatively immune. After the redness of the erythema and the increased skin temperature, following the exposure, has subsided, the skin may become "tanned" (the pigment increased). Subsequent exposures produce less and less erythema while the tanning increases. The skin is then said to acquire "protection." Miescher has recently shown from histological studies that "protection" against sunlight consists mainly of a thickening of the corneum layer of the skin. This is the outer horny layer of the skin and as it is known that the penetration of ultra-violet rays is

small, a thickening of the outer layer would prevent the ultra-violet rays reaching the deeper sensitive layers.

Hausser and Vahle have shown that erythema is produced by ultra-violet rays at the extreme short wavelength limit of the sun spectrum, in fact, the same region of the sun's spectrum which has the antirachitic properties. Erythema may also be produced by ultra-violet lamps, in which case the maximum erythema occurs at a wavelength about 3000 Å. The erythema-producing property of the spectrum decreases from 3000 Å until a wavelength of 2800 Å is reached. The erythema then increases again until 2500 Å is reached, after which it appears to decrease with shorter wavelengths, according to Hausser. Uhlmann has studied the pigmentation resulting from different parts of the ultra-violet spectrum. Pigmentation produced by 3000 Å reached its maximum two days after irradiation and persisted for fifteen days and perhaps longer. Pigmentation produced by 2500 Å, reached a maximum after three days and then decreased. Waves of 3130–3660 Å produce pigmentation. It seems fairly well established that ultra-violet radiation penetrates but a short distance into the skin. The percentage penetration for different wavelengths as given by Hasselbach and substantiated by later observers, is contained in the following table:

Wavelength Å		4360	4050	3660	3340	3130	3020	2970	2890
Thickness of skin	{ 0.1 mm	59	55	49	42	30	8	2	0.01
	{ 1.0 mm	0.5	0.3	0.08	0.02	0	0	0	0

The different layers of the skin have slightly different absorption coefficients, or abilities to absorb radiation, as determined by Bachem. Certain infra-red radiation on the other hand is quite penetrating, according to Dorno and Danforth. Danforth finds a maximum penetration at 11,500 Å, which becomes very small at 5400 and 1500 Å. Rays in the red and near infra-red are, therefore, particularly useful when it is desired to heat the skin and the first centimeter of subcutaneous tissue. The increased temperature in the capillaries (up to 45°) may contribute to a more rapid destruction of certain bacterial toxins, according to Sonne. An ordinary light bulb of high voltage has a maximum intensity in the near infra-red and should be particularly well suited for treatment of this kind. Roentgen rays and gamma rays have a

very important medical application. The hard, or short wavelength roentgen and gamma rays, are very penetrating and have the important property of destroying or inhibiting the growth of the rapidly growing cancer cells while the normal tissue cells are affected to a less degree. Excessive doses of roentgen rays cause injurious effects, hence it is necessary to regulate the dosage as already mentioned. Excessive doses, which are finally fatal to animals, produce at first an increase and later a decrease of the lymphocytes in cats, according to Wright and Bulman. The polymorphonuclear leucocytes also decrease after a preliminary rise. The effect on tissues varies with the dose, the distribution of the radiation, and the type of tissues.

Bodenstein: *Z. phys. Chem.* 85:329 (1913); *Chem. Rev.* 7:215 (1930).

Clark: *Am. J. Physiol.* 69:200 (1924).

Eidinow: *Brit. Med. J.* 2:160 (1927).

Fricke and Morse: *Am. J. Roent. Rad. Ther.* 18:426 (1927).

Hardy: *Am. J. Hyg.* 7:811 (1927).

Hausser and Vahle: *Strahlentherapie* 13:41 (1921).

Hill: *Rapp. Conf. Int. Lumière, Lausanne-Leysin* (1928).

## PART III

### ORGANIC

In the introduction we considered enzymes as catalysts speeding up changes in organic compounds. The reader is probably familiar with the action of digestive enzymes in the hydrolysis of foodstuffs, but in addition to these, many enzymes produced by bacteria cause chemical changes in the gut. Furthermore, the enzyme action of yeast on sugar is very similar to the transformation of sugar in muscle tissue. Hence the study of fermentation and putrefaction has a direct bearing on enzyme action in relation to the human body, and it seems logical to consider fermentations as a whole before proceeding to the more difficult problems of higher organisms; in fact, it seems logical to consider a whole group of plant products — not only those of yeasts, molds, and bacteria, but also those of higher plants — before considering those of animals. We may head this section as follows:

#### DIVISION 1

#### FERMENTATION PRODUCTS AND ESSENTIAL OILS

##### PARAFFIN SERIES

A number of paraffins have been shown to be produced by plants from other organic compounds. The most common of these is **methane** (marsh gas). It occurs as bubbles in marshes due to micro-organisms. It is also produced in the gut of higher animals. At the Pennsylvania State Agricultural College, Armsby built a calorimeter admitting a cow. The heat produced by the cow was directly measured. The methane produced in the gut of the cow (which would ordinarily have been lost) was carried out by means of a rubber tube and burned as a gas jet day and night, and the heat determined. Some organisms can utilize paraffins as sources of energy, thus *Bacillus methanicus*, *Mycobacterium*, *Methanomonas*, *Pseudomonas*, and *Penicillium* utilize methane as a source of carbon and energy in the presence of oxygen. Some higher paraffins are obtained from beeswax, gedda, or East Indian wax.

Gascard and Damoy: Compt. rend., 177:1442 (1923).

## ALCOHOL SERIES

The alcohols of the methyl series act as anesthetics, the addition of one carbon atom increasing the anesthetic (intoxicating) power three times. The toxicity of the alcohols on lower organisms follows this same rule, according to Traube; but in relation to humans, whereas the first effect may be as with lower organisms, the metabolic products of these alcohols differ. By metabolism is meant the sum total of chemical changes that a compound undergoes in the body after absorption from the gut or other modes of entrance.

**Methanol** is said to have caused blindness in as small a quantity as 10 cc. The great toxicity of methanol is supposed to be due to the fact that it is not metabolized at a rapid rate, the narcotic effect lasting from 3 to 4 days, and intermediate products, formaldehyde and formic acid, being produced for a long time. The fatal dose by mouth is between 100 and 250 cc. or breathing air containing more than 0.2%. Considerable quantities are excreted unchanged by the lungs, and *formic acid* is found in the urine. Methanol is oxidized by some micro-organisms. See allyl alcohol.

Autenrieth: Arch. Pharm. 258:1 (1920).

Sollmann: J. Am. Med. Assoc. 63:915 (1914).

**Ethyl alcohol** is produced during muscular contraction and occurs in normal tissue, blood 0.004%, liver 0.0026%, brain 0.004% (Gettler), and when drunk is metabolized rapidly in the body so that the amount excreted in the urine and breath is only a few per cent. It yields 7 Cal. per g., but this energy cannot be used for muscular work. A dose of 45 cc. decreased sensitivity and lengthened reflex time in man; the hypnotic dose is 45-60 g. Deep narcosis results when the concentration in the blood reaches 0.2%. Ethyl alcohol is produced by fermentation of sugar by an enzyme, zymase, in yeast. See "Co-enzyme." Harden and Young showed that the addition of phosphates increased the alcoholic fermentation of sugar by zymase, but the mechanism is poorly understood. Hexose phosphate and diphosphate are formed but the addition of glucose phosphate to yeast-culture did not increase the fermentation whereas it increased fermentation by zymase. Formation of alcohol is thought to be due to a Cannizzaro reaction, i.e., the formation of one molecule of alcohol and one of acid out of two molecules of aldehyde.

Iodates, bromates, and other salts increase alcoholic fermentation by yeast in the presence of oxygen but these are usually considered to be ingredients of yeast-food rather than as having to do with the enzyme action. Arsenate greatly increases the rate of fermentation, according to Harden.

Cirrhosis of the liver has been attributed to ethyl alcohol, although Mallory suggests that it is due to the copper from the copper still in which the alcohol was distilled. The lethal dose of alcohol is uncertain owing to its rapid combustion in the tissues. B. Fischer found only 54.1 g. in the tissues of a man who had died, probably of acute alcohol poisoning, but Juckenack found about six times as much.

Benedict: J. Am. Med. Assoc. 66:1424 (1916).

Fühner: Deut. med. Wochschr. 58:1041 (1932).

Gettler, Niederl and Benedetti-Pichler: J. Am. Chem. Soc. 54:1476 (1932).

Juckenack: Z. Untersuch. Nahrungs und Genussmittel 16:742 (1908).

Mellanby: Med. Research Comm. Special Rept. 31:1 (1919).

Propyl alcohol is produced in small quantities in ethyl alcohol fermentation by yeast. It may be utilized by mycobacteria and pseudomonas. It is three times as narcotic ("*intoxicating*") *per molecule* as ethyl alcohol and is said to be only three times as *toxic*. It is about twice as effective *per gram* as ethyl alcohol. The literature on the physiology of propyl alcohol is very meager, however. Micro-organisms oxidize it to propionic acid, and this action probably occurs to a small extent in the gut.

Propyl alcohol not only may occur in alcoholic drinks but also is added to food as a preservative.

Baldwin: Am. J. Physiol. 56:127 (1921).

— and Harrison: Proc. Am. Physiol. Soc.; Am. J. Physiol. 59:453 (1922).

Isopropyl alcohol is utilized by mycobacteria and pseudomonas. It is also produced by micro-organisms. Sixteen cubic centimeters daily shows no toxicity, but 23 cc. produces a narcotic effect. The toxicity is supposed to be less than with propyl alcohol. It has been used to anesthetize cats by the stomach-tube, the effect lasting hours or days.

Grant: J. Lab. Clin. Med., 8:382 (1923).

Butyl alcohol is nine times as intoxicating as ethyl alcohol per mol. It is produced by micro-organisms, and butyl alcohol fermentation is of industrial importance.

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It is used in separating organic compounds by their solubility. Dakin found that, by continuous extraction of a protein hydrolysate with butyl alcohol, the feebly ionized amino acids and proline were quantitatively removed, whereas the dicarboxylic and diamino acids remained behind. Foster used it for extracting thyroxine.

Dakin: Biochem. J. 12:290 (1918).

Isobutyl alcohol is produced by micro-organisms. It is used in the purification of morphine.

Franzen: Arch. exptl. Path. Pharmacol. 133:111 (1928); 135:118 (1928).

Amyl alcohol is produced by micro-organisms and is 27 times as intoxicating as ethyl alcohol per mol. This term is sometimes used to include certain isomeres of which *d*-amyl alcohol and isoamyl alcohol have been isolated from fermentations.

Duceschi: Arch. ital. biol. 70:93 (1920).

Isoamyl alcohol is the constituent of fusel oil which is chiefly responsible for its powerful intoxicating effect. Whiskey is distilled and much of the fusel oil is allowed to remain in the still. That which passes over is adsorbed by the charcoal of a barrel which is charred inside.

Blume: Arch. exptl. Path. Pharmacol. 110:46 (1925).

*n*-Hexyl alcohol, *n*-octyl alcohol and secondary *n*-hendecatyl alcohol occur as esters in higher plants. A number of higher alcohols occur as esters in waxes. They are *pisangceryl*, *cetyl*, *octadecyl*, *eikosyl*, *carnaubyl*, *ceryl*, *myricyl*, *cocceryl*, *psyllosteryl*, and *tarchaunyl* alcohol.

Oppenheimer and Pincussen: Tabulae Biologicae (Berlin) 3:34, 40 (1926).

**Allyl alcohol.** The toxicity of wood alcohol has been attributed to its content of 0.2–0.5% allyl alcohol, 0.5 cc. causing the death of a dog in 7 hours. Some may be gotten rid of by vomiting as it has a powerful emetic property. It has been used in chemical warfare, 5 parts of its vapor per million of air producing irritation and 50 parts death in 30 days. One cubic centimeter produces 362 cc. of vapor. Five- to six-tenths per cent in blood prevents putrefaction for 5 or 6 days.

Atkinson: J. Pharmacol. Proc. 25:144 (1925).

McCord: J. Am. Med. Assoc. 98:2269 (1932).

## ALDEHYDE SERIES

**Formaldehyde**,  $\text{HCHO}$ , has been considered as an intermediate in the formation of sugar in green leaves in the sunlight. Baly isolated it from a colloidal water-solution of nickel carbonate acted on by ultra-violet rays. This was denied by other workers, and Baly later concluded that impurities in their solutions reversed the reaction. There has been some prolonged discussion as to whether formaldehyde occurs in green leaves. Klein and Weiner precipitated formaldehyde in green leaves with "dimedon" (dimethyldihydroresorcinol) and in this way caused its accumulation. This was confirmed by Pollacci and Bergamaschi in 1929. This action is probably similar to that of the precipitation of acetaldehyde by means of bisulfite except that the resorcinol compound is colored and may be detected in the leaf itself.

Formaldehyde is toxic. A 12-kg. dog tolerated 1 g. daily in the diet. If the solution is strong it causes erosion in the alimentary canal. Recovery of man has been reported after taking 60 cc. of a 40% solution, and fatal cases all took over 90 cc. Some is oxidized in the tissues to formic acid.

Baly: *Ind. Eng. Chem.* 16:1016 (1923); *J. Am. Med. Assoc.* 101:1736 (1933).

Barton and Pratt: *Biochem. J.* 24:1210 (1930).

Klein and Weiner: *Biochem. Z.* 168:340 (1926).

Pollacci and Bergamaschi: *Atti accad. Lincei* 10:687 (1929).

Sommer, Bishop, and Otto: *Plant Physiol.* 8:564 (1933).

**Paraformaldehyde.** If formaldehyde is allowed to stand in aqueous solution, paraformaldehyde is formed by condensation of 3 molecules of the former. It has been administered to animals for the purpose of methylation of various compounds in the body. If fed with arginine, creatine is produced. If the solutions in which it is formed are allowed to stand longer and are alkaline, sugar is eventually produced. The lethal dose is 90 g.

Glickman and Vanderkleed: *J. Am. Pharm. Assoc.* 2:958 (1925).

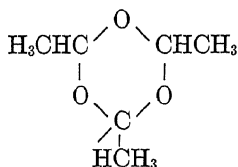
Thompson: *Biochem. J.* 11:307 (1917).

**Acetaldehyde**,  $\text{H}_3\text{CCHO}$ , is very widely distributed in nature, occurring in petroleum, plants, and animals. It is supposed that it is an intermediate product in the formation of fatty acids from sugars in the body, being transformed first into acetic acid. In the test tube, however, acetaldehyde condenses into  $\beta$ -hydroxy-

butyraldehyde. The beating heart was shown to produce 6 mg. acetaldehyde per 100 g. of tissue per hour by the "dimedon" test. Perfusion through the liver results in a Cannizzaro-reaction, in which 2 molecules of aldehyde form 1 of ethyl alcohol and 1 of acetic acid. It is said to increase after drinking alcohol, in which case the blood may contain 1.6 mg. per 100 cc. and the urine nearly 1 mg. per 100 cc.

Knoop and Jost: *Z. physiol. Chem.* 141:55 (1925).

**Paraldehyde.** Three molecules of acetaldehyde polymerize to form one of paraldehyde.



It is used as a hypnotic, 100 g. showing no toxicity whereas 3-15 cc. per hour produces hypnosis. The lethal dose is supposed to be 150 cc.

Fee: *J. Pharmacol.* 34:305 (1928).

**Methyl glyoxal**, pyruvaldehyde,  $\text{CH}_3\text{COCHO}$ , is supposed by some to be an intermediate in sugar metabolism. On enolization methyl glyoxal will have a hydroxyl group in alpha position to a carbonyl group which is a requisite for a compound to be a sugar. It gives crystalline derivatives with phenylhydrazine or substituted phenylhydrazine, which may serve for identification. By this method methyl glyoxal as well as pyruvic acid has been shown to be an intermediate in yeast fermentation of sugar by Neuberg and Kobel. Glyoxalase changes methyl glyoxal to lactic acid. This was shown to take place in glycolysis of animal tissues by Vogt in 1929. Apozymase from yeast converts hexose diphosphate into methyl glyoxal.

Vogt: *Biochem. Z.* 211:17 (1929).

**Butyraldehyde**,  $\text{CH}_3(\text{CH}_2)_2\text{CHO}$  occurs in oil of eucalyptus.

Guerin and Lormand: *Compt. rend.* 170:1589.

**Aldol**,  $\beta$ -hydroxybutyraldehyde,  $\text{CH}_3\text{CHOHCH}_2\text{CHO}$ , is interesting as it is formed by the condensation of 2 molecules of

acetaldehyde, a process which is, therefore, called the aldol condensation. Fricke found small quantities in the urine of diabetics. Aldol may be dehydrated to form crotonic aldehyde, which may be detected by dimethyldihydroresorcinol (dimedon).

Aldol has been suggested as an intermediate between sugar and fatty acids. The acetaldehyde condenses to aldol, which is oxidized to an acid. More units of acetaldehyde may be added to the chain. Knoop found that fatty acids are broken down in the body 2 carbon atoms at a time, so they are probably built up in the same way. Liver destroys aldol,  $\beta$ -hydroxybutyric acid being formed.

Fricke: Z. physiol. Chem. 116:126, 129 (1921).

Isovaleraldehyde, *n*-caprylaldehyde, *m*-pelargonaldehyde, and *n*-capric aldehyde occur in essential oils.

Soden and Rojahn: Ber. 34:2809 (1901); Perfumers J. 9:13 (1926).

Glyoxal,  $\text{CHO} \cdot \text{CHO}$ , is oxidized in the liver to glycollic acid. Glyoxalase converts glyoxal and ammonia into glycine, an amino acid. Glyoxal and ammonia on standing will unite, and this in turn will unite with formaldehyde dissociated from glyoxal to form imidazole, a ring compound found in several biological compounds of great importance.

Schaffer and Friedmann: J. Biol. Chem. 61:585 (1924).

$\alpha$ - $\beta$ -Hexenic aldehyde,  $\text{H}_3\text{C}(\text{CH}_2)_2\text{CH}:\text{CHCHO}$ , occurs in green plants.

Curtius and Franzen: Sitzber. Heidelberg Akad. Wiss. (1910).

## KETONE SERIES

Acetone,  $\text{CH}_3\text{COCH}_3$ , arises in the body by decarboxylation of acetoacetic acid by an enzyme (decarboxylase). The normal concentration in blood is 0.3 mg. per 100 cc. and in urine 0.46–6.24 mg. per 100 cc. It is increased in diabetics and may then be noticeable in the breath. Haldane stated that the administration of  $\text{NaHCO}_3$  (0.6 g. per kg.) increases the acetone output.

An increase in acetone in the body is called ketosis though that term includes the acetone precursors,  $\beta$ -hydroxybutyric acid and acetoacetic acid. Ketosis is most severe in diabetes mellitus, though mild ketosis may occur in starvation, early in phosphorus poisoning, during anesthesia, in some infections, and sometimes

on a fat diet. This question will be considered again under  $\beta$ -hydroxybutyric acid.

It is said to cause cataracts by direct action on the crystalline lens of the eye. Guinea-pigs survive for 2.5 months with a daily injection of 0.1 g. Two grams is fatal to rabbits in 6 minutes.

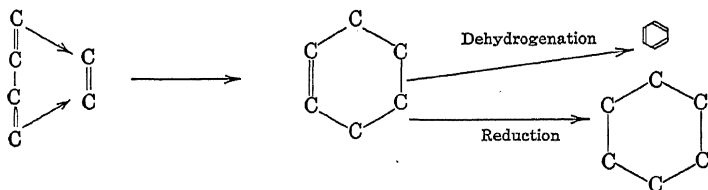
Griggs and Schaffer: J. Biol. Chem. 48:413 (1921).

Hubbard and Wright: Clifton Springs Med. Bull. (1923-1926); J. Biol. Chem. 61:377 (1924); Ann. Clin. Med. 3:634 (1925); Proc. Soc. Expt. Biol. Med. 19:91 (1921).

### TERPENE SERIES

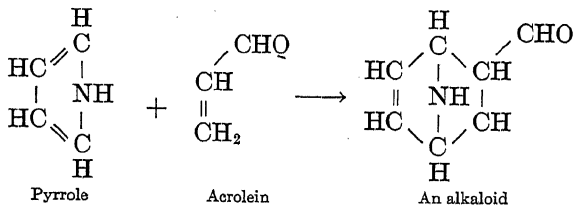
A terpene is a hydrocarbon of the general formula  $C_{10}H_{16}$  or one-half (hemiterpene), one and one-half (sesquiterpene), or two or more (polyterpene) times this formula. Terpenes occur in essential oils, resins, or other vegetable aromatic products.

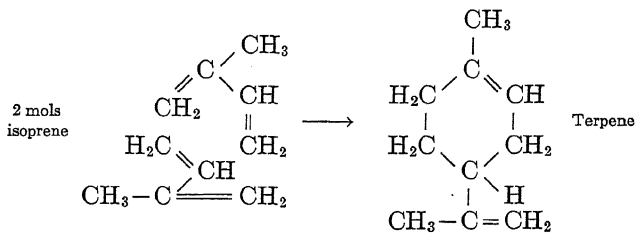
Probably the most important properties of isoprene are due to the presence of a conjugate system of double bonds. Diels and Alder have shown that a compound having a conjugate system of double bonds will add spontaneously to a compound having an active double bond.



The reaction requires no catalyst, proceeding at room temperature in the laboratory. It is very probable that this reaction is the basis for the formation of many complex molecules found in plants and animals, such as alkaloids and terpenes.

Examples:

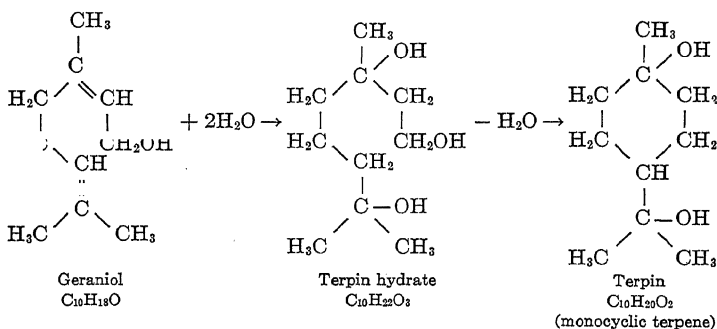




**Isoprene**,  $\text{C}_5\text{H}_8$ ,  $\text{HC} \begin{array}{c} \text{CH}_3 \\ | \\ \text{C} \\ || \\ \text{H}_2\text{C} \end{array} = \text{CH}_2$ , is a hemiterpene in that it is

half the formula of the terpenes. It polymerizes to terpenes, polyterpenes, and finally to a substance similar to rubber. It is said to produce cancer in mice.

Terpene derivatives may contain oxygen, that is, a hydroxyl or carbonyl group; and some of these are called camphors. They usually contain a 6-carbon ring. Terpin hydrate, a branched-chain compound, may by dehydration form terpin, a ring compound:



Geraniol and terpin hydrate are branched-chain compounds although most of the terpenes have a 6-carbon ring owing to union of the side chain of terpin hydrate with another point in the chain. Terpin hydrate lessens the cough-reflex in doses of 0.07-1 g.

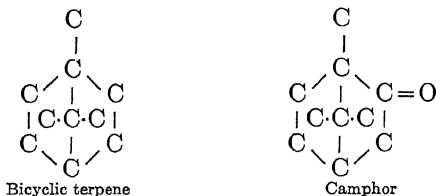
Graevenitz: Arch. exptl. Path. Pharmacol. 104:289 (1924).

**Limonene** is said to cause a fatty degeneration of the liver.

Graevenitz: Arch. exptl. Path. Pharmacol. 104:289 (1924).

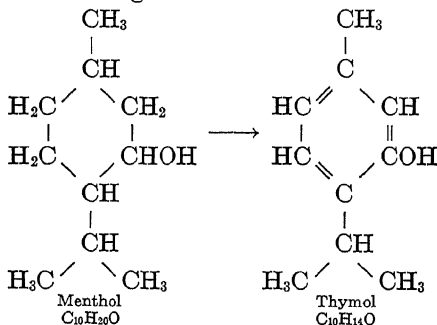
**Terpinene** causes the formation of methemoglobin. The terpenes and camphors are used as anesthetics.

**Bicyclic Terpenes.** **Camphor**  $C_{10}H_{16}O$  is a ketone of a bicyclic terpene.



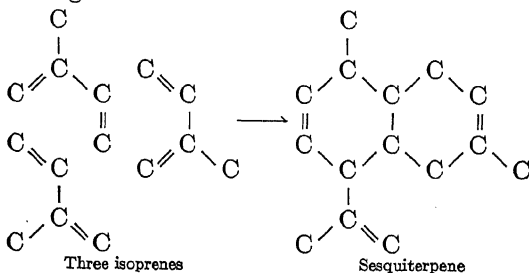
Burgers: Nature 118:116 (1926).

**Menthol**, mint-camphor,  $C_{10}H_{20}O$ , is an alcohol derivative of **menthene** found in *mint* and stimulates the cold nerve endings. It may be transformed into **thymol**, thyme-camphor,  $C_{10}H_{14}O$ , which is a benzene compound. This is just one instance of the transformation of open-chain compounds such as geraniol into closed-chain compounds such as the terpenes and finally into the unsaturated 6-carbon ring which is the most stable condition.



Steinle and Kahlenberg: J. Biol. Chem. 67:425 (1926).

In the following formulas the H atoms are omitted:



Just as 2 molecules of isoprene may form 1 of a terpene, so 3 molecules of isoprene may form 1 of:

**Sesquiterpene**, such as:

**Cadinene**,  $C_{15}H_{24}$ , which may be transformed into cadilene,  $C_{15}H_{32}$ , which is a naphthalene derivative.

**Santonin** is a lactone of a sesquiterpene of the eudesmol group and is used to rid the intestine of roundworms (*Ascaris*).

Stary: Arch. exptl. Path. Pharmacol. 133:192 (1928).

**Diterpenes**,  $C_{20}H_{32}$ , may be obtained by the distillation of resins.

**Triterpenes**,  $C_{30}H_{48}$ , or their alcohol derivatives, occur in elemi resin.

The terpenes were used by the Egyptians in embalming and occur in foods, in which case they are considered as flavors or perfumes. They are detoxicated in the body by conjugation with glycuronic acid or other substances.

**Sapogenins** are grouped with the triterpenes.

Rubber has been estimated to be formed of 1000 isoprene units or more — some estimate a molecular weight of 300,000.

Ruzicka: Ann. Rev. Biochem. 1:581 (1932).

## STEROL SERIES

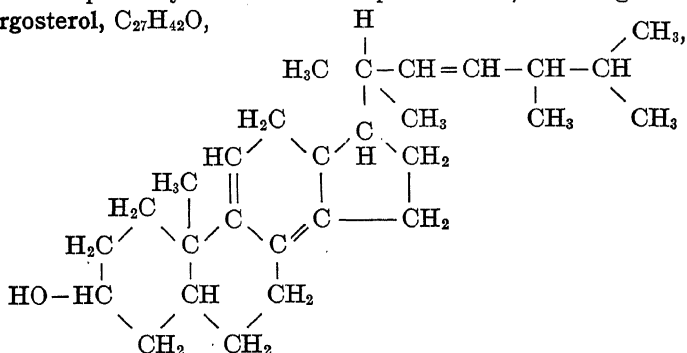
**Sterols** (solid alcohols) are supposed to be oxidation products of triterpenes,  $C_{30}H_{48}$ , in fact, the formula for **stigmasterol** (found in seeds of various plants) is  $C_{30}H_{48}O \cdot H_2O$ .

The chief plant sterol is **sitosterol**,  $C_{27}H_{42}O$ , which is widely distributed in plants.

Cowell: Brit. Med. J. I:594 (1925).

Schönheimer showed that sitosterol is not absorbed by animals, and that is probably true of all other plant sterols, including:

**Ergosterol**,  $C_{27}H_{42}O$ ,



found in yeast and ergot, except that ergosterol when irradiated by ultra-violet light of about 3000 Å undergoes an isomeric change and is then absorbable (Schönheimer). Two antirachitic isomers of ergosterol, having conjugate double bonds, have been isolated from irradiated ergosterol by Windaus and Linsert.

Häussler and Brauchli: *Helv. Chim. Acta*, 12:187 (1929).

Heilbron, Morrison and Simpson: *J. Chem. Soc.* 1933:302 (1933).

Schönheimer, v. Behring, and Gottberg: *Z. physiol Chem.*, 208:77 (1932).

**Irradiated ergosterol** (called by the American Medical Association **viosterol**) is physiologically identical with a substance derived from natural sources, vitamin D, and greatly economizes the



FIG. 34. Caries in the teeth of a polynesian who died before advent of the white man. Chappell.

calcium-phosphate metabolism. It occurs in animal fats; therefore Eskimos and Sioux Indians eating a diet high in animal fat have good bones and teeth. Vitamin D is most concentrated in cod-liver oil. Nowadays the synthetic product is taken. Mae Mellanby claims that the feeding of viosterol to children greatly improves the teeth. It also improves the bones as it prevents rickets and osteoporosis. Pickerill thought that Polynesian aborigenes had good teeth but this is disputed (fig. 34).

There seems to be a correlation between sunshine and good bones and teeth (figs. 35, 36) which would indicate that irradi-

ation of ergosterol in the skin is necessary with present food habits.

Bills: Chem. Rev. 3:425 (1927).

Rosenheim and Webster: Biochem. J. 20:537 (1926).

Windaus and Holtz: Naturwiss. (Math.-Phys. Kl.) 217 (1927).

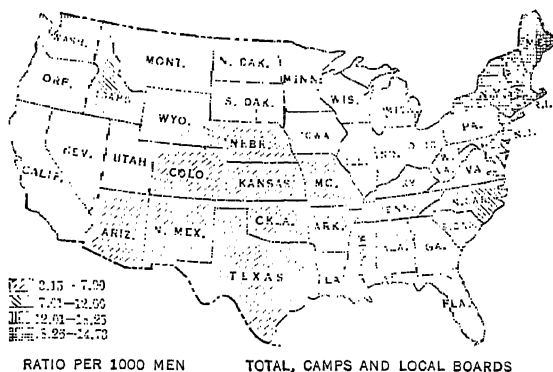


Fig. 35a. Geographical distribution of defective teeth. Defects found in drafted men. Government Printing Office.

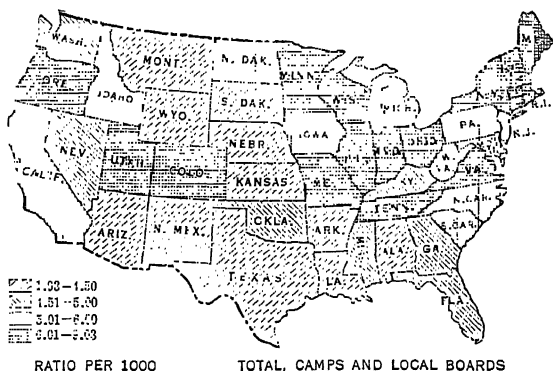
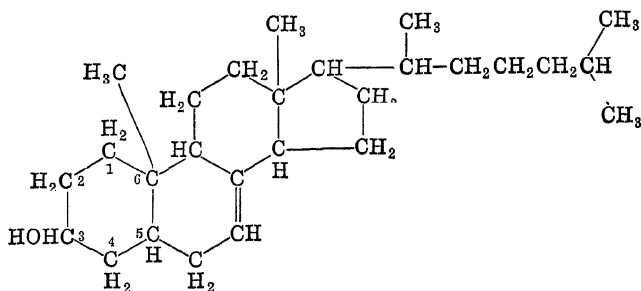


Fig. 35b. Curvature of the spine. Government Printing Office.

**Cholesterol**,  $C_{27}H_{45}OH$ , is found in every living animal cell but chiefly in the bile, and it forms gall-stones. The structural formula is not exactly known, but the studies of Rosenheim, Wieland, and Windaus make the following formula probable:



The numbering of the carbon atoms is not the same as that used by Schönheimer. The cholesterol content of the white matter of the nervous system is 2.5%, of the pons and medulla 4%, and of the

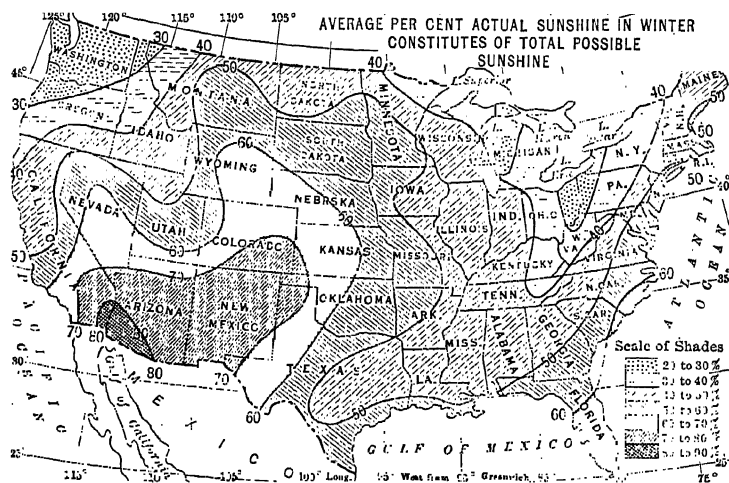


FIG. 36. Hours of sunshine in the United States.

spinal cord about the same percentage. Dried liver contains 6%, dried human muscle 0.5%, and blood serum 1-3 mg. per cc. Unlike plant sterols, it may be absorbed from the alimentary tract, and when eaten in too large quantities, it is deposited in the arteries (atherosclerosis). It is contained in the covering of the plasma membrane of living cells and influences their permeability. It is, therefore, concerned with hemolysis. This is a special case of cytolysis or destruction of the plasma membrane of the cell.

When the plasma membrane of the red blood cell is destroyed, many substances diffuse out, including the hemoglobin, which

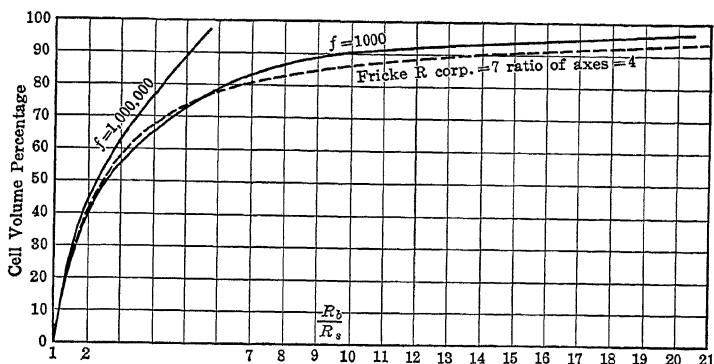


FIG. 37. Ratio of impedance of blood to impedance of serum. Journal of Biological Chemistry.

can easily be seen. Cholesterol neutralizes hemolytic poisons, such as saponin and tetanolysin. The high electrical resistance of the cell surface may be explained by assuming a superficial covering of cholesterol (figs. 37, 38).

Eckstein and Wile: J. Biol. Chem. 69:181 (1926).

Randles and Knudson: J. Biol. Chem. 66:459 (1925).

A slight isomeric change determines whether the sterols are absorbed or not absorbed; thus *cholesterol* is absorbed whereas *allocholesterol*, which differs only in the reversal of a carbon atom is not absorbed.

**Dihydrocholesterol**, which is a saturated derivative of cholesterol, is absorbed, whereas **koprosterol**, which differs only in one asymmetric carbon atom, is not absorbed. Koprosterol, therefore, is excreted in the feces

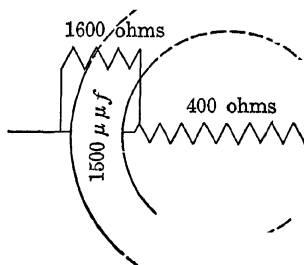


FIG. 38. Diagram of specific dielectric and conductivity characteristics of plasma membrane or cell surface of the erythrocyte. The capacity in micro-micro-farads of the plasma membrane is given together with its resistance in ohms, as parallel circuits. The resistance of the cell interior is given in ohms in series with the plasma membrane. J. B. C.

since it is formed from other sterols in the alimentary canal by the action of micro-organisms.

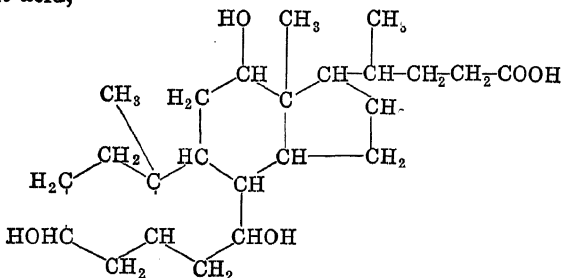
Therefore Schönheimer thinks that the difference between ergosterol, which is not absorbed, and viosterol, which is absorbed, is in the same asymmetric carbon atom as above. Cholesterol is absorbed from the food and also synthesized in the animal body and is the intermediate in the synthesis of ergosterol. Irradiation of the body will affect the ergosterol in the skin and thus produce the same effect as eating viosterol.

Schönheimer: *Science* 74:579 (1931); *Z. physiol. Chem.* 192:73, 77, 86, 93, 97 (1930).

#### BILE ACID SERIES

Bile acids are related to the sterols.

Cholic acid,



having 3 hydroxyl groups as well as the carboxyl group, is more soluble than cholesterol; in fact, it increases the solubility of fatty acids. It occurs in bile as the sodium salt.

Hammarsten: *Z. physiol. Chem.* 43:127 (1904).

**Deshydroxycholic acid**, lacking the upper hydroxyl group, also occurs in bile as the sodium salt.

Schönheimer: *Biochem. Z.* 147:258 (1924).

**Choleic acid** is a combination of deshydroxycholic acid and a fatty acid. It is soluble in water. Bile makes fatty acid soluble, perhaps because of choleic acid formation. If the bile is prevented from passing into the gut the fats are digested but not absorbed.

Broun: *Proc. Soc. Exptl. Biol. Med.* 23:596 (1926).

Bile acids are conjugated with glycine and taurine (which we will take up under the proteins) to form:

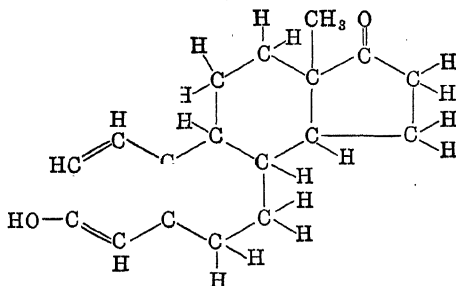
**Glycocholic acid and**

Still: Am. J. Physiol. 88:729 (1929).

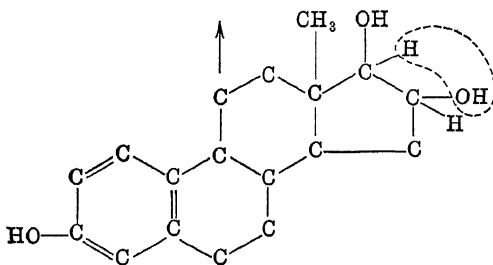
**Taurocholic acid.**

Rosenthal: J. Pharmacol. 25:449 (1925).

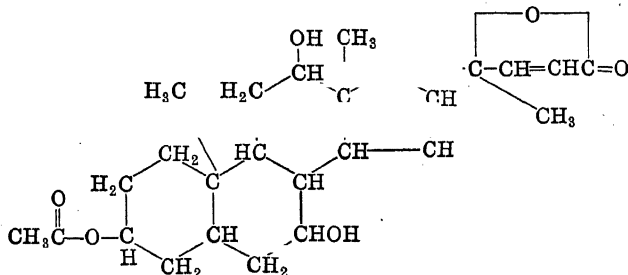
**Theelin** (see p. 43) is an estrogenic hormone.



**Theelol** is biologically like theelin.

**TOAD POISONS**

**Bufotalin** (derived from **bufotoxin** from parotid glands of toad).  
The position of the acyl and double bond is not certain.





**Xanthophyll**,  $C_{40}H_{56}O_2$ , is closely associated with carotin from which it is formed by oxidation, and both are found with chlorophyll in plants. The centers of its absorption bands are 4470 and 4795 Å in alcohol.

Palmer: J. Biol. Chem. 23:261 (1915).



FIG. 39. Rat with xerophthalmia from lack of vitamin A.

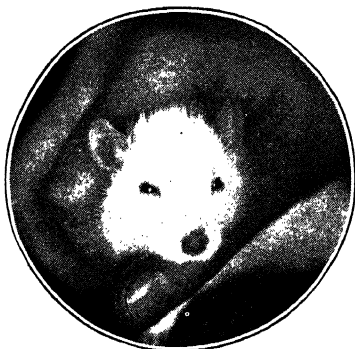
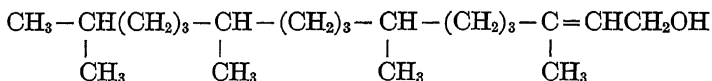


FIG. 40. Recovery on a carotin-containing food, dried spinach. McClendon and Schuck.

**Phytol** is an alcohol which is found combined with the porphyrin nucleus in chlorophyll.



Fischer and Löwenberg: Ann. 475, 183 (1929).

Javillier: Baude and Levy-Lajeunesse: Bull. soc. chim. biol. 7:39 (1925).

#### AROMATIC ESSENTIAL OILS

Essential oils contain quite a number of benzene derivatives; many of them are related to the terpenes, such as:

***p*-Cymene**,  $(\text{CH}_3)_2\text{CH}-\text{C}_6\text{H}_4-\text{CH}_3$ , which may be produced by dehydration and reduction of geraniol.

**Ascaridol**, according to Wallack's formula, is an oxidation product of *p*-cymene. It is used as a vermifuge and is the active principal of American wormwood.

Bert: Bull. Soc. Chem. 37:1252 (1925).

**Tyrosol**,  $\text{HO}-\text{C}_6\text{H}_{10}-\text{CH}_2\text{CH}_2\text{OH}$ , is a by-product of yeast fermentation, being derived from tyrosine, and is also produced in the gut.

Onslow and Robinson: *Biochem. J.* 19:420 (1925).

**Phenol** and **Cresol** (most of the cresol is *p*-cresol) are produced in the gut. Folin and Denis showed that they are largely (30-90%) excreted in the urine in the free form. In the herbivora they are largely conjugated with glycuronic acid. In man, conjugation is chiefly with sulfuric acid. The lethal dose of phenol is 0.5 g. per kg. for a dog and is 8.5-60 g. for man. Hirsch states that *m*-cresol is three times as antiseptic and one-quarter as toxic to man as phenol. Subcutaneous lethal dose, g. per kg. rabbit, is *m*-cresol 0.5, *o*-cresol 0.45, *p*-cresol 0.3, phenol 0.5. Their concentration in normal blood is 1-2 mg. per 100 cc.

Benedict and Theis: *J. Biol. Chem.*, 36:95 (1918).

Folin and Denis: *J. Biol. Chem.*, 22:309 (1915).

Hirsch: *Dental Summary*, 36:143 (1916).

**Catechol**, **resorcinol**, and **hydroquinone**, as well as **phloroglucinol** and **pyrogallol** are reducing substances found in wood-tar and are used in photographic developers as well as occasionally in antiseptic solutions.

Autenrieth: *Detection of Poisons and Powerful Drugs*, sixth edition, Blakiston's, Philadelphia (1928).

**Hexyl-resorcinol**,  $\text{CH}_3(\text{CH}_2)_4\text{CH}-\text{C}_6\text{H}_3(\text{OH})_2$ , a synthetic product (Johnson and Hodge), is used as a urinary antiseptic. Doses of 1.58 g. per kg. per day were harmless to white rats.

Leonard and Feirer: *J. Pharmacol.*, 28:395 (1926).

Poe, Fehlmann, and Johnson: *Univ. Colo. Bull. Studies*, 20:159 (1933).

**Quinone**, a derivative of **hydroquinone**, is interesting in that the combination of the two is used for the **quinhydrone** electrode for hydrogen-ion determination. A platinum wire is dipped into quinhydrone and placed in the solution to be tested. The quinhydrone dissociates into an equal number of molecules of quinone and hydroquinone, and the potential of the liquid is determined

by the hydrogen-ion concentration. The quinhydrone electrode is standardized by comparison with the hydrogen electrode.

Kolthoff: *Rec. Trav. Chim.* 42:186 (1923).

**Guaiacol** is interesting in that it is derived from guaiac resin, but it is not the chromogen of the guaiac resin. Guaiac resin is used as an indicator of an oxidase.

Muberly: *Lancet* 216:437 (1929).

**Toluene, benzyl alcohol, benzaldehyde, benzoic acid, phenyl acetic acid, and hydroxyphenylacetic acid**, all are derived from plants or fermenting liquids. The benzoic acid in cranberries preserves them until Christmas.

Sollmann: *Pharmacology*, second edition, Saunders, Philadelphia (1914).

**Homogentisic acid**, 2,5-dihydroxyphenylacetic acid, occurs in alkapton urine. The urine turns black on exposure to air, which may be due to the oxidation of homogentisic acid. It is said to be derived from tyrosine, an amino acid, with an intermediate quinoid form, a reaction catalyzed by an enzyme from beet-root.

Lieb and Lanyar: *Z. physiol. Chem.* 182:218 (1929); 181:199 (1929).

**Salicyl alcohol (saligenin), salicylaldehyde, and salicylic acid**, as well as **acetyl salicylic acid**, are used in medicine as analgesics and local anesthetics.

Jensen and Hirschfelder: *J. Pharmacol.* 24:423 (1925).

Stockman: *Edinburgh Med. J.* 34:396 (1927).

## DIVISION 2

### LIPIDES

#### FATTY ACID $C_nH_{2n}O_2$ SERIES

**Formic acid**,  $HCOOH$ , occurs in and derives its name from ants. It is found in the urine after drinking methanol. Four-tenths cubic centimeter per kilogram interferes with growth. Fresh meat extracts contain 0.5% calculated on the dry basis. It increases during putrefaction. An injection is very painful and leads to local infiltrations. *Normal blood is said to contain 10 mg. per 100 cc.* Its sodium salts injected intravenously cause diuresis.

Dakin and Wakeman: *J. Biol. Chem.* 9:329.

**Acetic acid** is produced chiefly by the oxidation of ethyl alcohol in the presence of oxygen by micro-organisms. Macfadyen, Nencki, and Sieber studied a woman with a fistula near the iliocecal valve, and for one month collected all the intestinal contents. The contents were practically always acid. The distillate of 2 kg. contained 1.5 g. of volatile fatty acids, and although these workers supposed this to be acetic acid entirely, it probably contained some propionic.

Acetic acid has been found in sweat and bile. When perfused through the liver it is changed finally to acetoacetic acid, but according to Thunberg an intermediate stage is succinic acid. It is stated that insulin is necessary for its complete combustion in the body.

According to Thunberg, acetic acid is an intermediate stage in the destruction of both sugar and fat and therefore of some of the protein in the body. In this way the metabolism of fats and sugars comes together at one point and proceeds from that point on.

The laxative or cathartic effect of roughage in the diet is chiefly due to the stimulating action of acetic acid (produced from the roughage by fermentation) on the secretions and contractions of

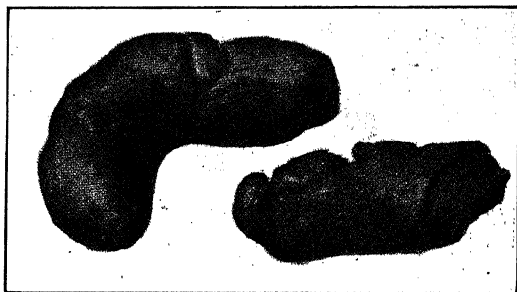


Fig. 41. The normal stool. E. L. Gardner.

the gut. It is the accumulation of these secretions (in addition to 50% of the cellulose that is unattacked) that gives the stimulus to the rectum and produces the normal stool (fig. 41). The increased secretion of mucus has been observed even in the stomach. A definite laxative effect was noted in a man on drinking 10 cc. of glacial acetic acid partly neutralized with sodium hydroxide and dissolved in 200 cc. of water. When given in large quantities to

dogs, it acts as a violent purge (Lusk). An excess acetic fermentation in the gut may cause the colon to become spastic (fig. 42) and cause the spastic stool (figs. 43, 44) and give rise to symptoms

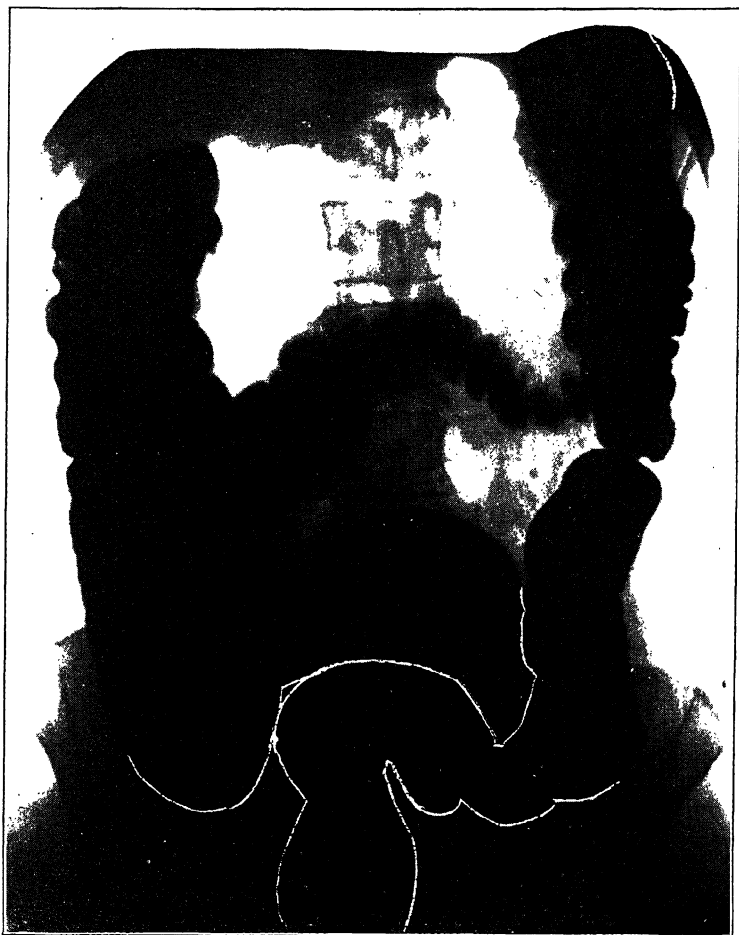


FIG. 42. Spastic colon, X-ray positive.

portrayed in fig. 45. A deficiency of acetic fermentation may cause constipation (fig. 46).

Lusk showed that it is not changed to sugar in a diabetic dog,

although when given with sugar it causes a marked increase in metabolism of the normal dog. It is very rapidly oxidized to carbon dioxide and water in the body.

Macfadyen, Nencki, and Sieber: Arch. exptl. Path. Pharmacol. 28:318 (1891).

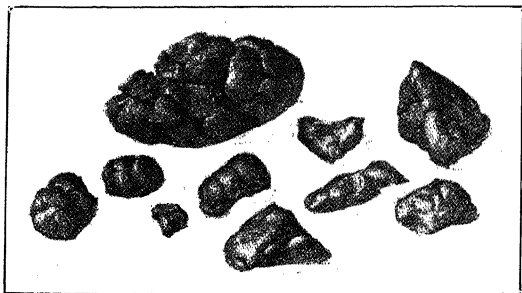


FIG. 43. The spastic stool. E. L. Gardner.

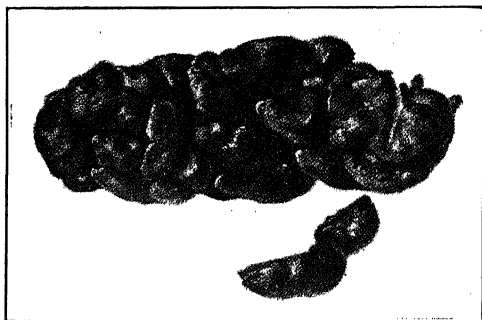


FIG. 44. The baled-hay type of spastic stool. E. L. Gardner.

**Propionic acid**,  $\text{H}_3\text{CCH}_2\text{COOH}$ , is miscible in all proportions with water, from which it may be salted out as an oil, thus the name given it by Dumas, which indicates "the first fatty acid." It is entirely changed to sugar in the diabetic dog. Lactic (and perhaps pyruvic) acid is an intermediate product.

It has been isolated from secretions from rheumatics and detected in eczematous exudates. The lethal dose given intravenously is about 0.5 g. per kg. rabbit.

It is found in equal quantities with acetic acid in the feces of calves. A cereal diet produces more than a whole milk diet.

It is said to cause a depression of the motility of the stomach but it stimulates the intestine. Probably one effect of roughage is



FIG. 45. The spastic gut as portrayed by Cruikshank.

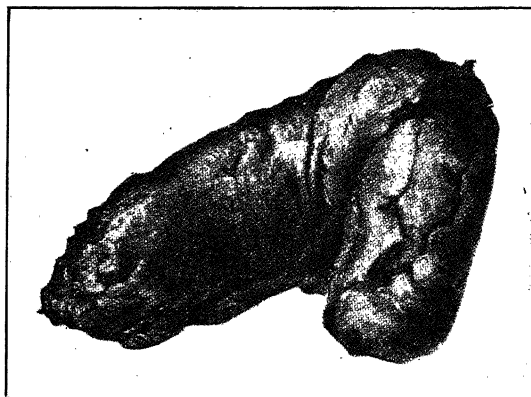


FIG. 46. The constipated stool.. E. L. Gardner.

due to some propionic acid produced from it by fermentation in the gut, and it is probable that in some analyses it was not separated from acetic acid but was reported as acetic.

Dickinson and Watson: *J. Pharmacol.*, 34:65 (1928).

**Butyric acid**,  $\text{H}_3\text{C}(\text{CH}_2)_2\text{COOH}$ , has the odor of rancid butter and is found in sweat, feces, and urine. It is the longest chain fatty acid miscible in all proportions with water and is the shortest chain that occurs in animal fats. Macallum supposes that its high solubility leads to its rapid excretion on a relatively high fat diet.

One-third gram per kilogram rabbit is fatal on intravenous injection. It stimulates the contraction of the intestine, but it is stated that the concentration must be below 0.04%. Since this is a high concentration, however, it is clear that any butyric acid which arises from the fermentation of roughage in the gut stimulates it and in this way reinforces the effect of the acetic and propionic acid similarly formed.

It is oxidized to  $\beta$ -hydroxybutyric and  $\beta$ -ketobutyric (acetoacetic) acids in the body, and these are normally broken down to acetic acid, according to Knoop. Snapper showed that this last step may occur in the kidney, and when the kidney is overloaded with butyric or when it is damaged, as it is in some diabetics,  $\beta$ -hydroxybutyric and acetoacetic acids pass out in the urine. The latter may be decarboxylated to acetone. Foods that give rise to butyric acid are called "ketogenic" foods; foods which do not give rise to butyric acid, since they furnish fuel for the body and so avoid the necessity of burning the ketogenic foods, are called "antiketogenic."

Knoop showed that fatty acids are burned 2 carbon atoms at a time by  $\beta$ -oxidation and splitting off of acetic acid, and, therefore, all fatty acids with an even number of carbon atoms give rise to butyric. In the diabetic, antiketogenic foods are ineffective because they are transformed into sugar that cannot be burned. Thus all fatty acids with an odd number of carbon atoms give rise to propionic acid and are therefore antiketogenic. Butyric acid may arise from proteins in putrefaction.

Knoop: Beitr. Chem. Physiol. Path. 6:150 (1904).

Macallum: Can. Med. Assoc. J. 22:3 (1930).

Schaffer: J. Biol. Chem. 47:433 (1921).

Snapper and Grünbaum: Biochem. Z. 201:464, 473 (1928); 181:418; 185:223 (1927).

Watanabe: Arch. Jap. Med. 35:381 (1928).

## KETOGENIC FATTY ACIDS

(even number of C atoms) all except acetic yield butyric acid

<i>Acid</i>	<i>Derivation of name</i>	<i>Formula</i>
Acetic.....	Vinegar.....	$\text{H}_3\text{CCOOH}$
Butyric.....	Butter.....	$\text{H}_3\text{C}(\text{CH}_2)_2\text{COOH}$
6 Caproic } .....	Goat (fat).....	$\text{H}_3\text{C}(\text{CH}_2)_4\text{COOH}$
8 Caprylic } .....		$\text{H}_3\text{C}(\text{CH}_2)_6\text{COOH}$
10 Capric } .....		$\text{H}_3\text{C}(\text{CH}_2)_8\text{COOH}$
12 Lauric.....	Laurel.....	$\text{H}_3\text{C}(\text{CH}_2)_{10}\text{COOH}$
14 Myristic.....	Nutmeg.....	$\text{H}_3\text{C}(\text{CH}_2)_{12}\text{COOH}$
16 Palmitic.....	Palm (oil).....	$\text{H}_3\text{C}(\text{CH}_2)_{14}\text{COOH}$
18 Stearic.....	Solid.....	$\text{H}_3\text{C}(\text{CH}_2)_{16}\text{COOH}$
20 Arachidic.....	Peanut.....	$\text{H}_3\text{C}(\text{CH}_2)_{18}\text{COOH}$
22 Behenic.....	Ben (oil).....	$\text{H}_3\text{C}(\text{CH}_2)_{20}\text{COOH}$
24 { Carnaubic.....	Carnauba (wax)....	$\text{H}_3\text{C}(\text{CH}_2)_{22}\text{COOH}$
{ Lignoceric.....		$\text{H}_3\text{C}(\text{CH}_2)_{22}\text{COOH}$
26 Cerotic.....	Wax.....	$\text{H}_3\text{C}(\text{CH}_2)_{24}\text{COOH}$
28 Octacosanic (montanic) (28)	Montan wax from brown coal.....	$\text{H}_3\text{C}(\text{CH}_2)_{26}\text{COOH}$
30 Mellisic.....	Honey.....	$\text{H}_3\text{C}(\text{CH}_2)_{28}\text{COOH}$

## ANTI-KETOGENIC FATTY ACIDS

(odd number of C atoms) yield propionic acid which is oxidized to lactic and condensed to glycogen

1 Formic.....	Ants.....	$\text{HCOOH}$
3 Propionic.....	First (fat).....	$\text{H}_3\text{CCHCOOH}$
5 Valeric.....	Valerian.....	$\text{H}_3\text{C}(\text{CH}_2)_3\text{COOH}$
7 Enanthic.....	Enanthus.....	$\text{H}_3\text{C}(\text{CH}_2)_5\text{COOH}$
9 Pelargonic.....	Pelargonium.....	$\text{H}_3\text{C}(\text{CH}_2)_7\text{COOH}$
11 Undecylic.....	11.....	$\text{H}_3\text{C}(\text{CH}_2)_9\text{COOH}$
13 Ficocerylic.....	Ficus.....	$\text{H}_3\text{C}(\text{CH}_2)_{11}\text{COOH}$
15 Pentadecylic.....	15.....	$\text{H}_3\text{C}(\text{CH}_2)_{13}\text{COOH}$
17 Margaric.....	Pearl.....	$\text{H}_3\text{C}(\text{CH}_2)_{15}\text{COOH}$

Fatty acids form monomolecular films on water-air surfaces, with the COOH group in the water, and hence the cross-sectional area of the molecule may be determined.

**Soap** is a salt of a fatty acid. Castile soap is almost pure sodium oleate. Palm-oil soap is mainly sodium palmitate.

Sodium ricinoleate will form a clear solution in water because of the presence of OH groups, increasing the solubility in water.

Soaps of the lower fatty acids (salt water soap) are soluble in salt (sea) water.

Calcium and magnesium soaps of ordinary fatty acids are in-

soluble, but calcium and magnesium soaps of linseed oil are soluble in water. Calcium soap floats, is lighter in weight than potassium soap because only 1 molecule of calcium unites with 2 molecules of fatty acids, and the molecular weight of calcium is less than that of potassium. Ordinary floating soaps contain air bubbles.

Soaps are emulsifying agents as they form monomolecular films on oil-water surfaces, reducing surface tension.

Sodium soaps stabilize emulsions of oil in water, and calcium soaps stabilize emulsions of water in oil (Clowes). Butter is an emulsion of water in oil stabilized by the calcium of the butter-milk. The atom of sodium (or potassium) is diametrically thicker than the carbon chain of the fatty acid and forms a wedge with the large end, the sodium, in the water and the small end, the carbon chain, in the oil. The calcium atom is narrower than 2 carbon chains, and the small calcium end is in the water. Although the reversal of an oil-water emulsion stabilized with sodium soap by the addition of  $\text{Ca}^{++}$  and mechanical agitation has been used to explain changes in permeability of the plasma membrane of the living cell, it should be remembered that the plasma membrane is a solid structure (see p. 145).

Clowes: J. Phys. Chem. 20:407 (1916).

#### $\text{C}_n\text{H}_{2n-2}\text{O}_2$ SERIES

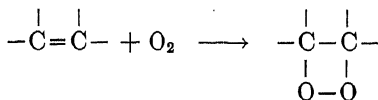
**Crotonic acid**,  $\text{H}_3\text{CCH}=\text{CHCOOH}$ , occurs in croton oil. When taken by mouth it is irritating, causing violent purgation. In common with other unsaturated acids, cis-trans isomerism occurs. The melting-point of these acids increases with the increase of carbon atoms in the chain but is much lower than that of the saturated acid of the same number of carbon atoms.

Friedman: Hofmeister's Beitr. 11:365 (1908).

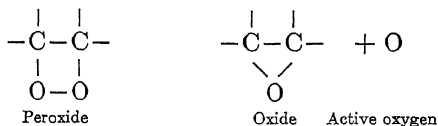
**Oleic acid**,  $\text{H}_3\text{C}(\text{CH}_2)_7\text{CH}$ , is widely distributed in nature.



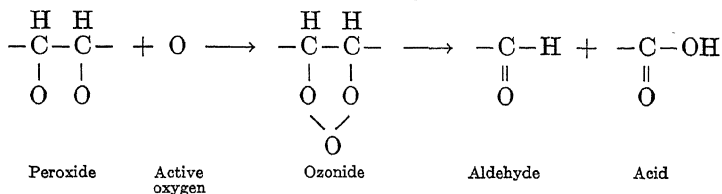
The trans isomere, elaidic acid, occurs in hydrogenated fats. Oleic acid is easily oxidized at the double bond, forming a peroxide:



This may decompose, liberating active oxygen:



The active oxygen may unite with another peroxide, forming an ozonide, and then decompose, forming an aldehyde and an acid:



The bad taste and smell of "rancid" fat is due to the aldehyde. The aldehyde formed may polymerize, thus "drying" the oil. Rancidity is due to the production of aldehyde and acid of shorter chain. This oxidation is catalyzed by light after a latent period, but on second exposure to light no latent period occurs (called an analog of "memory" by Mathews).

The presence of the double bond in oleic acid is indicated by the "iodine number," which is the percentage of iodine that would unite with it if two atoms of iodine united at the double bond. Two atoms of iodine are too large to unite at the double bond, but one atom of iodine and one of bromine or one of iodine and one of chlorine may unite, and the iodine number may be calculated from the quantity of IBr or ICl combined.

Bloor: J. Biol. Chem. 67:33 (1926).

Mathews: Physiological Chemistry, fifth edition, 75, 609, William Wood & Co., N. Y. (1930).


**Nervonic acid**,  $\text{H}_3\text{C}(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_{13}\text{COOH}$ ,  $\text{C}_{24}\text{H}_{46}\text{O}_2$ , is found in the cerebroside, nervon, and in the phosphatide, sphingomyelin.

Klenk: Z. physiol. Chem. 145:244 (1925); 166:268, 287 (1927).

### CHAULMOOGRIC SERIES

(Occur in chaulmoogra oil and are used to cure leprosy)


Owing to the irritating effect of the oil on the alimentary tract, ethyl esters of the acids are injected intramuscularly.

**Hydnocarpic acid**, -CH<sub>2</sub>(CH<sub>2</sub>)<sub>9</sub>COOH. The lethal dose of ethyl hydnocarpate is 0.5 g. per kg. rabbit.

Hollmann and Dean: J. Cut. Dis., 37:367 (1919).

Perkins: J. Philippine I. Med. Assoc., 5:369 (1925).

Power, F. B.: Am. J. Pharm., 87:493 (1915).

**Chaulmoogric acid**, -CH<sub>2</sub>(CH<sub>2</sub>)<sub>11</sub>COOH, kills acid-fast bacteria in dilutions of 1/100,000, of the sodium soap. Dose of ethyl ester 1-5 cc. intramuscularly per week, or the same dose by mouth.

Rogers, L.: Int. Med. Gaz. 54:165 (1919); Lancet, 1:1178 (1921).

Shriner and Adams: J. Am. Chem. Soc. 47:2727 (1925).

#### C<sub>n</sub>H<sub>2n-4</sub>O<sub>2</sub> SERIES

(Two double bonds)

**Linolic acid**, H<sub>3</sub>C(CH<sub>2</sub>)<sub>4</sub>CH:CHCH<sub>2</sub>CH:CH(CH<sub>2</sub>)<sub>7</sub>COOH, comes from linseed oil. It oxidizes more easily than oleic and forms aldehydes, which condense (drying of paint). It is found in the body.

Its calcium soap is soluble in water.

It has an anti-enzyme action against trypsin (Jobling). This action is caused by adsorption, coating the substrate with a monomolecular film of fatty-acid molecules. The more double bonds in the molecule the greater the area covered, as the fatty-acid molecules stand on end but are folded at the double bond (cis-form).

Jobling and Peterson: J. Exptl. Med. 19:239 (1914).

#### C<sub>n</sub>H<sub>2n-6</sub>O<sub>2</sub> SERIES

(Three double bonds)

**Linolenic acid**, H<sub>3</sub>CCH<sub>2</sub>CH:CHCH<sub>2</sub>CH:CHCH<sub>2</sub>CH:CH(CH<sub>2</sub>)<sub>7</sub>COOH, occurs in linseed oil. Its calcium soap is soluble in water. Linolenic acid is hemolytic when 0.2 g. per day is injected into a rabbit.

Burr showed that 100 mg. of its glyceride daily prevented fat-deficiency in a rat, or cured the lesions in the skin and kidneys caused by fat deficiency.

RETARDATION OF PEPTIC ACTIVITY  
Coagulated ovalbumin 5%, fatty acid 4%

Acid added	Amino <i>N</i> mg. per 100 cc.	Area covered by mole- cule in monomo- lecular film
Caproic acid.....	6.6	—
Palmitic acid.....	5.7	21
Oleic acid.....	2.8	46
Linolenic acid.....	1.3	65

Velluz: Bull. soc. chim. biol. 9:483 (1927).

$C_nH_{2n-8}O_2$  SERIES

**Clupanodonic acid**,  $C_{18}H_{28}O_2$ , is derived from fish oil.

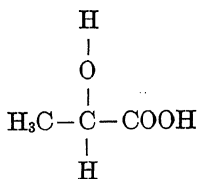
Tsujimoto: J. Chem. Ind. Japan 26:1013 (1923).

**Arachidonic acid**,  $C_{20}H_{32}O_2$ , is found in liver.

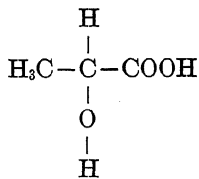
Levene and Rolfe: J. Biol. Chem., 51:285 (1922).

HYDROXY ACIDS,  $C_nH_{2n}O_3$  SERIES

**Lactic acid**, because of having an asymmetric carbon atom, occurs in dextro and levo forms:



*d*-Lactic acid



*l*-Lactic acid

Some organisms burn up one isomere and leave the other.

*d*-Lactic acid is produced by and is found in every tissue of the body. Lactic acid is changed to glycogen in the liver, a process which prevents its passage into the urine except in severe exercise. Normal blood contains 21 mg. per 100 cc., but its value may reach 100 mg. after 100 minutes' running. Meigs showed that excised muscle developed equimolecular proportions of lactic and phosphoric acids. Lohman claims that adenosine-triphosphoric acid, inorganic phosphate, and magnesium are necessary as co-ferments. Meyerhof studied this phenomenon during muscular contraction and found that part of the lactic acid dis-

appeared in the resting phase without oxygen and claimed that the remainder was burned when oxygen was admitted. The evidence on which this is based is as follows:

Hill showed that energy liberated in muscular contraction may be divided into two main parts, *action* and *restitution*. The action energy may be divided again, first that which occurs on the contraction following nerve stimulation, and secondly, energy (heat) which is liberated gradually on relaxation. In other words, if the muscle contracts in the absence of oxygen, energy is liberated at once, and then there is a slow liberation of energy for some time afterwards, the total being action energy. If oxygen is admitted at this point, there is another liberation of energy and this is restitution energy. This restitution energy is due to the oxidation of lactic acid in the muscle. It is produced in about one second after the single muscle twitch. (Gerard measured action and restitution heat of a nerve on stimulation; *both* are produced in the *absence of oxygen*.)

The restitution energy is about the same amount as the contraction energy, and only this contraction energy may be transformed into useful work and only by adjusting the load during the contraction so that the optimum load is always being raised by the muscle. This is very difficult to accomplish, and usually the efficiency of muscular work is less than 25% instead of being 50%. This may be put down in calories. In the production of 1 g. of lactic acid from glycogen in the muscle, 0.370 Cal. of heat are produced. If the change took place in a calorimeter, only 0.230 Cal. of heat would be produced. The difference is due to the combination of the lactic acid with the protein. Now 0.230 Cal. is due to hydrolysis and 0.140 is due to the combination with protein, and the sum makes up the 0.370 total. Some of this lactic acid is burned about one second later, but 3.836 Cal. are liberated on the burning of 1 g. of lactic acid. So even in the presence of oxygen, the total energy is not liberated. Why is that? The only conclusion that Hill could draw was that part of the lactic acid is built back into glycogen or glucose diphosphate. In the burning of  $\frac{1}{2}$  g. of lactic acid, one whole gram of glucose is hydrolyzed to lactic acid and  $\frac{1}{2}$  g. of lactic acid is built back into glucose diphosphate. (In nerve  $10^{-7}$  cal. per gram is *action* and  $10^{-6}$  total heat = action + *restitution heat*.)

That does not indicate the mechanism of the muscle contrac-

tion, however. Some models have been made which are suggestive. If a violin string be immersed in a solution of lactic acid, it will shorten and at the same time increase in volume, but all models are more or less defective. (The nerve impulse is associated with a colloidal change in the nerve surface and resulting release of ions.)

In running a hundred-yard dash, lactic acid is produced in larger amounts than can be immediately burned. So lactic acid accumulates in the body and is burned up when violent exercise ceases. (Lactic acid appears in nerve only in absence of oxygen.)

Muscular energy influences the metabolism. The metabolism may be raised by *extremely hard work* from 2000 to 12,000 Cal. for 24 hours. Five-sixths of the latter is due to muscular energy. So nearly all the metabolism of the lumberman is muscular metabolism. Hill has shown that the R.Q. of this extra (muscular) metabolism is unity. The muscular metabolism gives rise to lactic acid, and lactic acid is burned in the body. It is possible that glutathione influences that burning, taking 2 hydrogen atoms from lactic acid to give pyruvic acid. (The R.Q. in nerve is 0.95. Glutathione in nerve explains 3 hours' activity without oxygen.)

In violent exercise the lack of oxygen is great, and Hill has shown that as much as 130 g. of lactic acid may accumulate in the body. The muscles may contract in the absence of oxygen until, with a certain definite increase in lactic acid, the muscle becomes fatigued. The muscle cannot keep on contracting with accumulation of lactic acid. (A nerve cannot be fatigued in less than 3 hours even in absence of oxygen.)

Gerard: Science 66:495 (1927).

Hill: Physiol. Rev. 2:310 (1922).

Meyerhof and Lohman: Biochem. Z. 171:381 (1926).

Shaffer: Physiol. Rev. 3:419 (1923).

*L*-Lactic acid is the acid of sauerkraut and ensilage. The laxative action of roughage is partly due to its lactic acid fermentation. *L*-Lactic acid is produced by *B. levolactici*. It is rapidly converted into the racemic mixture by alkali, hence its use in metabolism may depend on racemization.

Meyerhof and Lohman: Naturwissenschaften, 14:196, 437 (1926).

**$\beta$ -Hydroxybutyric acid** is oxidized in the liver or kidney. Diabetics may excrete up to 100 g. per day. The liver contains

an enzyme, hydroxybutyrase, which catalyzes its oxidation to acetoacetic acid. The lethal dose injected intravenously is 1.59 g. per kg. rabbit.

Since  $\beta$ -hydroxybutyric acid is an oxidation product of butyric acid, the work of Knoop, Snapper, and Macallum on this subject is given under butyric acid.

Wilder and Winter: J. Biol. Chem. 52:2 (1922).

**Ricinoleic acid**,  $\text{H}_3\text{C}(\text{CH}_2)_5\text{CHOHCH}_2\text{CH}:\text{CH}(\text{CH}_2)_7\text{COOH}$ , from castor oil, is used in medicine. It is more soluble than oleic because of having an OH group. It is used as a purgative and to detoxify bacterial toxins. Mixtures of bacterial toxins and castor-oil soap have lower toxicity but the same antigenic effect when compared with the toxins alone. It likewise detoxicates rattlesnake venom. Some streptococci are killed by a dilution of 1/5000 in 7 hours at  $35^\circ$ .

Larson, Evans, and Nelson: Proc. Soc. Exp. Biol. Med. 22:194 (1924).

**Phrenosinic (cerebronic) acid**,  $\text{H}_3\text{C}(\text{CH}_2)_{21}\text{CH}(\text{OH})\text{COOH}$ , will combine with bacterial toxins in the same manner as ricinoleic. It occurs in phrenosin (cerebron), a cerebroside of the brain. It was called by Thudichum, neurostearic acid.

Klenk: Z. physiol. Chem. 174:214 (1928).

#### $\text{C}_n\text{H}_{2n}\text{O}_4$ SERIES

**Glyceric acid**,  $\text{HCOH}\cdot\text{HCOH}\cdot\text{COOH}$ , is said to be an intermediate product in alcoholic fermentation. A 0.01 *N* solution is toxic when perfused through frog's muscle.

Neuberg and Rubin: Biochem. Z. 67:77 (1914).

**Diphosphoglyceric acid**,  $\text{CH}_2 - \text{CH} - \text{COOH}$ , accounts for

$$\begin{array}{ccc} | & & | \\ \text{O} & & \text{O} \\ | & & | \\ \text{PO}(\text{OH})_2 & & \text{PO}(\text{OH})_2 \end{array}$$

30–42% of the acid-soluble phosphoric acid found in the blood, there being 12–20 mg. per 100 cc. Since it is hydrolyzed by kidney pulp, Jost thinks that it is a precursor of urinary phosphate.

Jost: Z. physiol. Chem. 165:171 (1927).

**Dihydroxystearic acid** occurs in oils and soils, making the latter infertile. It is responsible for part of the purgative effect

of castor oil. The positions of the hydroxyls influence the melting-point.

Upson and Powel: Ind. Eng. Chem. 7:420 (1915).

### $C_nH_{2n}O_7$ SERIES

Satvic acid is a purgative.

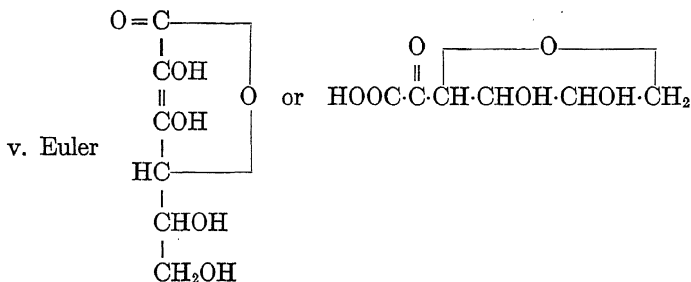
Zellner: Monatsh., 46:611 (1926).

Gluconic acid,  $H_2COH(CHOH)_4COOH$ , is formed in fermenting mixtures by the oxidation of glucose. Calcium gluconate is used in medicine as a safe means of injecting calcium into the blood.

Levene: J. Biol. Chem., 59:135 (1924).

Glycuronic acid,  $HCO(CHOH)_4COOH$ , occurs only as a glucoside, and the glycuronates are considered under the glucosides.

Ascorbic acid (vitamin C) was isolated by Szent-Györgyi. Levene and Raymond conclude that it is either



Levene and Raymond: Science 78:64 (1933).

### $C_nH_{2n-2}O_3$ SERIES

Glyoxylic acid is used in testing for tryptophan.

Fearson: Biochem. J., 14:548 (1920).

Pyruvic acid,  $H_3CCO_2COOH$ , yields glucose in the diabetic dog. No toxic symptoms follow injection of 8-13 g. subcutaneously into a dog. Lactic acid seems to be an intermediate in its transformation into glucose since *inactive* lactic acid has been found in the urine of rabbits receiving pyruvic acid and lactic acid appears on perfusing the liver with pyruvic. Thunberg believes pyruvic acid is decarboxylated to acetaldehyde in the body.

Aubel: Bull. soc. chim. biol. 6:345 (1924).

**Acetoacetic acid**,  $\text{H}_3\text{CCOCH}_2\text{COOH}$ , is excreted when much fat is metabolized. Normally only 3–15 mg. of acetoacetic acid + acetone is excreted in the urine per day. Its decarboxylation to acetone occurs in the body as well as in the urine.

Since muscles of depancreatized dogs utilize it as well as normal dogs its excretion is not a fundamental characteristic of diabetes but merely an index of the failure of a high fat metabolism.

Frogs deprived of their livers destroy it. Snapper believes that the chief seat of its destruction is the kidney.

Shaffer: J. Biol. Chem. 47:433 (1921).

**Levulinic acid**,  $\text{H}_3\text{CCO}(\text{CH}_2)_2\text{COOH}$ , is derived from fructose and from the sugar groups in animal nucleic acids and pseudo-mucin. When more than 6 g. is swallowed, some is excreted in the urine.

Kossel and Newman: Ber. 27:2215 (1894).

#### DICARBOXYLIC ACIDS, $\text{C}_n\text{H}_{2n-2}\text{O}_4$ SERIES

The *melting-point* of these acids of *even* number of carbon atoms is *high*; of those of odd number, *low*. The *water solubility* of these acids of *even* number of carbon atoms is *low*, and of *odd* number *high*.

In large doses, dicarboxylic acids are nephropathic or cause a pathology of the kidney when injected intravenously. They occur in living tissue.

**Oxalic acid**,  $\text{COOH}\cdot\text{COOH}$  (m.p.  $198^\circ$ , solubility 10%), is especially toxic owing to precipitation of calcium, 0.1% potassium oxalate preventing coagulation of blood. Normal urine contains 10–40 mg. per 100 cc. Some of this may arise from uric acid by hydrolysis of oxaluric acid, a peptide combination of oxalic acid and urea. It occurs in many vegetable foods, and death has occurred from eating rhubarb leaves, on account of the oxalic acid they contain.

Widmark: Acta. Med. Scand. suppl., 26:340 (1928).

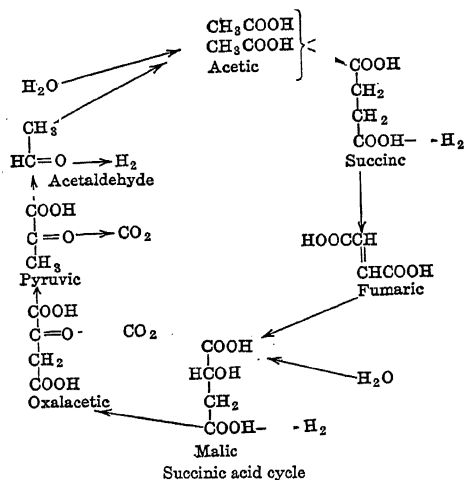
**Malonic acid**,  $\text{HOOCCH}_2\text{COOH}$  (m.p.  $135.6^\circ$ , solubility 74%). Muscle contains an enzyme, fumarase, which transforms fumaric into malonic acid.

Thunberg: Skand. Arch. Physiol. 22:430 (1911).

**Succinic acid**,  $\text{HOOCCH}_2\text{CH}_2\text{COOH}$  (m.p.  $185^\circ$ , solubility 7%). In a normal kidney the  $\beta$ -hydroxybutyric (or acetoacetic) acid formed from the ordinary fatty acids, is broken down into carbon dioxide and water. The exact steps have not been worked out, but according to Thunberg succinic acid is an intermediary product. Lactic acid is also broken down to carbon dioxide and water and perhaps in a similar manner.

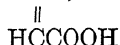
Wieland's theory of oxidation involves dehydrogenation, the hydrogen uniting with a hydrogen acceptor. Methylene blue can be used as the acceptor. The following experiment was performed by Thunberg to show the theory of dehydrogenation: Place muscle free from  $\text{H}_2$  donators (containing succinic acid dehydrogenase), in a tube, put in a substance to be oxidized (succinic acid) and methylene blue, and pump off the oxygen from the tube before closing it. The methylene blue will turn colorless owing to its union with the hydrogen that is given off by succinic acid dehydrogenation. On admitting oxygen, the tube turns blue again.

Thunberg developed the following working hypothesis: Acetoacetic acid is changed into acetic acid by hydrolysis in the kidney (Snapper). The acetic acid then undergoes the following changes:



$C_nH_{2n-4}O_4$  SERIES

**Fumaric acid**,  $HOOCCH=CHCOOH$ , is formed during alcoholic fermenta-



tation. It occurs in muscle and according to Thunberg is an intermediate in metabolism, being transformed by liver carboxylase into lactic acid and then into glucose. It can be used as source of carbon for *Penicillium glaucum*. Its isomere, **maleic acid**, is toxic to mammals and very little utilized by *Penicillium*.

Ahlgren: Compt. rend. soc. biol., 87:1409 (1922).

 $C_nH_{2n-2}O_5$  SERIES

**Malic acid**,  $HOOCCHOHCH_2COOH$ , is formed from fumaric acid by muscle pulp, and according to Thunberg is an intermediate in metabolism. The lethal dose of sodium malate is 1.5 g. per kg. rat and 3.5 g. per kg. rabbit.

Dakin: J. Biol. Chem., 52:183 (1922).

 $C_nH_{2n-4}O_5$  SERIES

**Oxalacetic acid**,  $HOCCOCH_2COOH$ , is said to arise in the body by dehydrogenation of malic acid. An enzyme from tissues, or even from yeast, decarboxylates it with the formation of pyruvic acid.

Ahlgren: J. Chem. Soc., 101:1570 (1913).

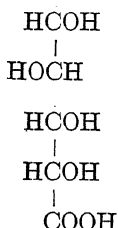
 $C_nH_{2n-2}O_6$  SERIES

**Tartaric acid** is used in baking powder. The nephrotoxic dose administered subcutaneously into rabbits is 0.25-1 g. The lethal dose for humans by mouth is 140-180 g. The tartrates are but slowly absorbed and hence stimulate the rectum by distension. The laxative dose is 0.5 g. tartaric acid or 10 g. Rochelle salts.

Leonard and O'Brien: J. Pharmacol. 28:109 (1926).

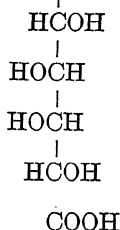
$C_nH_{2n-2}O_8$  SERIES

**Saccharic acid,**  $\text{COOH}$ , is formed by the oxidation of *d*-glucose and is very soluble in water. Baumgarten states that it is metabolized by diabetics.



Paderi: Arch. farmacol. sper. 22:96 (1916).

**Mucic acid,**  $\text{COOH}$ , is formed by the oxidation of *d*-galactose and is insoluble in cold water. Glucose and galactose may be distinguished by oxidation to these acids and observation of the precipitation of mucic acid.



Whittier: J. Am. Chem. Soc. 45:1391 (1923).

 $C_nH_{2n-4}O_7$  SERIES

**Citric acid** occurs in plants, especially citrus fruits, in blood serum (2.5 mg. per 100 cc.), and in milk (0.2%). About 73 mg. per day is excreted in sweat. Its most economical source is by fermentation of beet-sugar pulp. It is used to prevent coagulation in blood transfusion. Four grams intravenously was fatal to a 5.4-kg. dog. Its action seems to be the prevention of ionization of calcium. In blood transfusion slow intravenous or intramuscular injections are given. Blood pressure falls, and shock may result from overdose. The sodium component may cause edema.

Blood citric acid may be reduced in pneumonia, thrombosis, cancer, epilepsy, and parathyroid tetany. It may be increased in diabetes and nephritis.

Schultz: J. Lab. Clin. Med. 14:674 (1929).

Thunberg: Kung. Fysiograf. Sällsk. Lund Förhandl. 3:No. 17 (1933).

## ETHOLIDE SERIES

**Etholides** are described by Bougault and Bourdier as esters of an ordinary fatty acid and a hydroxy fatty acid.

## MONOHYDRIC ALCOHOL ESTER SERIES

These esters are found in essential oils. The longer the carbon chain the less the solubility in water.

C atoms	Name	Per cent soluble (20°)	C atoms	Name	Per cent soluble (20°)
2	Methyl formate	30	5	Ethyl propionate	2.3
3	Methyl acetate	25	5	Methyl butyrate	1.7
3	Ethyl formate	10	5	Isobutyl formate	1
4	Ethyl acetate	7.9	6	Ethyl butyrate	0.7
4	Methyl propionate	6.1	6	Butyl acetate	0.5
4	Propyl formate	2.8	6	Isoamyl formate	0.3
5	Propyl acetate	2.3	7	Amyl acetate	0.18

They are hydrolyzed by esterase and may be used to measure esterase activity. They are also hydrolyzed by lipase (fat-splitting enzyme). They have odor. Ethyl and propyl formates have a sweet taste.

V. Skramlik: Z. Sinnesphysiol. 56:69 (1924).

## GLYCERIDE SERIES

**Mono and diglycerides** of all the fatty acids of even number of carbon atoms are known. When not otherwise specified the triglycerides are understood. They are said to have two melting-points on account of some change taking place in heating.

**Triglycerides** have rarely been isolated from fats. The usual method of fat analysis is hydrolysis (saponification) and separation of the fatty acids. By this means it is impossible to determine whether triglycerides of a single fatty acid exist or whether they are mixed glycerides. Butyric acid is obtained by hydrolysis of butter, but butyric has such an intense taste that it is doubtful that it could exist in butter without being tasted. Probably mixed glycerides of butyric and higher fatty acids occur in butter.

Hydrolysis of fat is called saponification even when catalyzed by *lipase* in neutral solution without soap formation. The average molecular weight of fatty acids is indicated by the *saponi-*

Fat or oil	M.P.	I <sub>2</sub> No.	Saponifica- tion No.
Butter.....	33°	32	225
Beef.....	39	42	196
Pork.....	36	60	196
Human.....	18	62	196
Olive.....	6	84	191
Cottonseed.....	4	111	191
Cod-liver.....	4	156	190
Linseed.....	-27	188	190

fication number, the number of milligrams of KOH required to neutralize the fatty acids of 1 g. of fat. Oils from tropical plants have a lower iodine number than those from temperate-zone plants. When butter is saponified, fatty acids of even numbers (from 4 to 18) of carbon atoms are formed, 7% of which are volatile. Butter gets rancid quickly because it is an emulsion of water in oil. The water (buttermilk) contains bacteria, and the decomposition of butter is partly due to hydrolysis (made possible by the water and bacterial enzymes in droplets), liberating fatty acids. Oxidation occurs also (see oleic acid). Most fatty acids are insoluble in water but butter contains butyric, which is very soluble.

Alsberg and Taylor: *The Fats and Oils*, Stanford Univ. Press (1928).

**Tributyryn.** Rats will not eat a food mixture containing 5% of it. **Tricaproin** is tasteless.

Schizern: *Biochem. Z.* 195:96 (1928).

**Laurin**, m.p. 46.5°, is found in laurel oil.

Ozaki: *Biochem. Z.* 189:233 (1927).

**Palmitin**, m.p. 46° and 65.1°, predominates in palm oil and human fat.

Levene: *Physiol. Rev.* 1:327 (1921).

**Synthetic margarin**, under the name of **intarvin**, is fed to diabetics, but the commercial preparations have had a bad taste due to impurities. Whereas it is not ketogenic, it is not completely burned by the diabetic and must lead to the excretion of glucose or fatty acids.

Kahn: *Arch. Internal Med.* 35:44 (1925).

**Stearin**, m.p.  $54.5^{\circ}$  and  $70.8^{\circ}$ , is in low concentration in human fat but higher in beef tallow.

Bloor: Chem. Rev. 1:24 (1925).

**Olein**, m.p.  $-17^{\circ}$ , is the chief constituent of olive and winter-pressed cottonseed oil and is in high concentration in human fat. Its presence lowers the melting-point and raises the iodine number of the fat.

Leathes: Lancet 803, 853, 957, 1019 (1925).

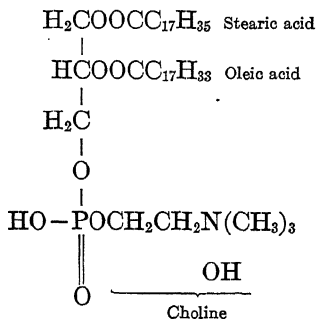
**Lipiodol** is iodized fat used for injection into lungs, blood vessels, and kidney-pelvis for X-ray shadows; when eaten, iodine is liberated.

**Linseed oil** is a mixture containing linolein and other unsaturated fats. Its iodine number varies and may be as high as 200. Burr found that white rats developed "eczema" on a fat-free diet but were cured by 1 drop of linseed oil per rat per day. Hansen cured eczema in children by adding linseed oil to the diet. The author studied for 10 years the diet of an adult with eczema and found that protein seemed to have no relation to it. Fermentable carbohydrates (for example pentosans, "roughage") made the symptoms worse. In avoiding fermentable (vegetarian) diets, meat diets were tried. The protein of the meat seemed not to be beneficial but the fat was beneficial. Linseed oil was finally tried. Ten cubic centimeters of linseed oil per day added to the diet was found to be beneficial. This study was complicated by the fact that dryness of the air made the eczema worse, so that humidifiers (air conditioners) had to be installed.

#### PHOSPHATIDE SERIES

##### MONOAMINO-MONO-PHOSPHATIDES

**Lecithin,**



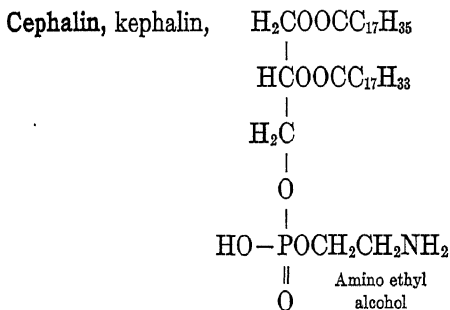
is hydrolyzed by lipase into fatty acids (one of which is oleic), glycerophosphoric acid, and choline. It is easily oxidized at the double bond of the oleic acid in the body, particularly in the liver, although cytochrome or some other iron catalyst is said to be necessary.

Lecithin aids in the solution of fat in bile. Cobra hemolysis is increased by lecithin. It is twice as abundant in corpuscles as in plasma.

Leathes supposes that lecithin is a stage through which fat passes in metabolism, and this is supported by Bloor. Dreschel, McCollum, and Halpin showed that lecithin is synthesized in chickens, and Dressoff indicated that it is synthesized in the liver.

Lecithin and cholesterol are constituents of the cell surface or plasma membrane, a discussion of which may be found under cholesterol, above. Pascucci made artificial membranes imitating the plasma membrane chemically and tested their permeability.

Bloor: J. Biol. Chem. 22:133 (1915).

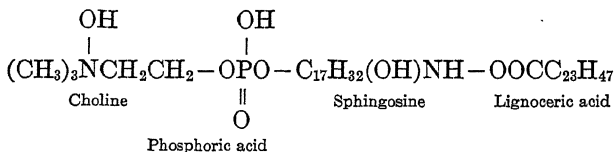


accelerates blood clotting and is a component of fibrin. It is separated from lecithin by adding alcohol to the ether solution, since cephalin is much less soluble than lecithin in alcohol. It was discovered in the brain by Thudichum but has been found elsewhere.

Levene and West: J. Biol. Chem. 16:419 (1915).

## DIAMINO-MONO-PHOSPHATIDE

## Sphingomyelin,



(name refers to marrow).

Anesthetics are soluble in these phosphatides, a fact which has been used in a theory of anesthesia by Overton and Meyer.

Levene: J. Biol. Chem. 15:153 (1915); 17:679; 18:453; 24:69; 26:69 (1916).

## DIVISION 3

## GLUCIDES

## SWEET ALCOHOL SERIES

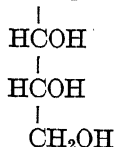
Glycol,  $\text{H}_2\text{COH}\cdot\text{CH}_2\text{OH}$  ("prestone") is formed by the decomposition of choline. It is not converted into glucose in the diabetic.

Dakin: Biochem. J. 3:57 (1909).

Glycerol,  $\text{H}_2\text{COH}\cdot\text{CHOH}\cdot\text{CH}_2\text{OH}$ , is a constituent of fats. It is a diuretic. It is converted quantitatively into glucose in the diabetic dog. *Bacillus welchii* converts it into acrolein at such a rate as to stop its own growth. It may be changed into acrolein,  $\text{H}_2\text{C}:\text{CHCHO}$ , by heating in the presence of a dehydrating agent; in fact, this is the ordinary test for glycerol and glycerides. It reacts with nitric acid to form nitroglycerin which lowers blood pressure.

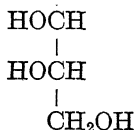
Catron and Lewis: J. Biol. Chem. 84:553 (1929).

Erythritol,  $\text{CH}_2\text{OH}$ , occurs in certain lichens.



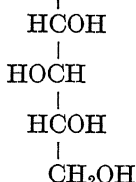
Bertrand: Compt. rend. soc. biol. 130:1330 (1900).

**Arabitol**,  $\text{CH}_2\text{OH}$ , can be utilized by certain soil bacteria and is found in many plants, including those used for ensilage.



Neuberg and Wohlgemuth: Z. physiol. Chem. 35:62 (1902).

**Xylitol**,  $\text{CH}_2\text{OH}$ , is an isomere of arabitol.

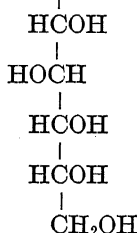


Bertrand: Bull. soc. chim. 5:554 (1891).

**Adonitol** is another isomere.

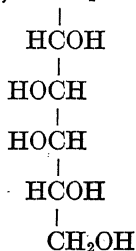
Mandel and Neuberg: Biochem. Z. 71:214 (1915).

**d-Sorbitol**,  $\text{CH}_2\text{OH}$ , occurs in mountain ash berries and other plants. When perfused through the diabetic liver, it is converted into sugar.

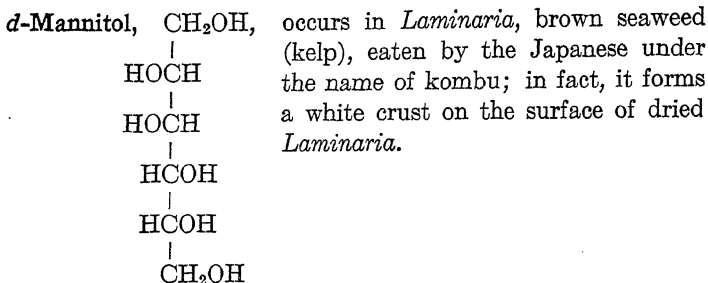


Davis, Slater, and Smith: Biochem. J. 20:268 (1926).

**d-Dulcitol**,  $\text{CH}_2\text{OH}$ , is an isomere of sorbitol.

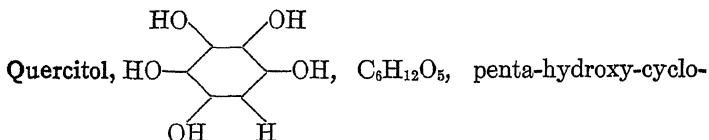


Uglow: Arch. Hyg. 95:89 (1925).



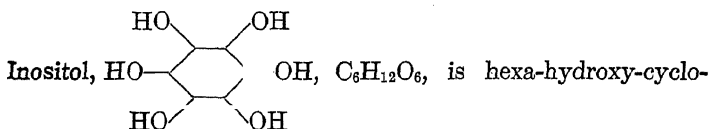
Evans: J. Am. Chem. Soc. 47:3085 (1925).

#### CYCLOSE SERIES



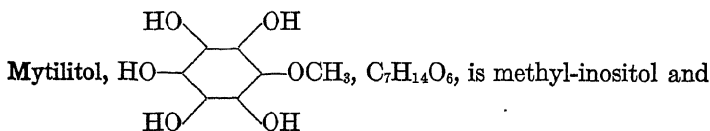
hexane, is found in trees and other plants.

Attree and Perkin: J. Chem. Soc. :234 (1927).



hexane. Muscle contains 0.003%. It is very abundant in green peas. Kulz gave a man 50 g. and recovered in the urine 0.5 g. It is thought to be eliminated in the feces. It is said to be "bios I," stimulating yeast growth.

Needham: Ergebnisse Physiol. 1:45 (1926).



is found in the edible sea mussel (*Mytilus edulis*).

McDowell: Proc. Soc. Exptl. Biol. Med. 25:85 (1927).

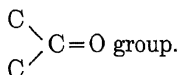
**Phytic acid** is inositol-hexa-phosphate and along with **phytin** which is its calcium salt is widely distributed in plants. It is

hydrolyzed by phytase. The phosphoric acid thus liberated is utilized by yeast in bread dough.

Anderson: J. Biol. Chem. 43:117; 44:429 (1920).

### MONOSACCHARIDE SUGAR SERIES

A monosaccharide, or simple sugar, contains one carbonyl group and has at least one hydroxyl group on a carbon atom that is  $\alpha$  to the carbonyl. Sugars containing 2 oxygen atoms are known as bioses; those containing 3, trioses; 4, tetroses; 5, pentoses; and 6, hexoses. Aldoses contain a  $-\text{CHO}$  group, and ketoses contain a



#### BIOSES

**Glycol aldehyde**,  $\text{H}_2\text{COH}\cdot\text{CHO}$ , is thought by some to be an intermediate product in the conversion of lactic acid and glycine to glucose and is itself converted into glucose when introduced into the body.

Greenwald: J. Biol. Chem. 35:461 (1918).

#### TRIOSES

##### Aldose

**Glyceric aldehyde**,  $\text{H}_2\text{COH}\cdot\text{CHOH}\cdot\text{CHO}$ , or glycerose, is converted into sugar in the body.

Sansum and Woodyatt: J. Biol. Chem. 24:327 (1916).

##### Ketose

**Dihydroxyacetone**,  $\text{H}_2\text{COH}\cdot\text{CO}\cdot\text{CH}_2\text{OH}$ , is converted into glucose in the body.

Mann, Bollmann, and Magath: Am. J. Physiol. 72:49 (1925).

#### TETROSES

##### Aldose

**d-Erythrose** is formed by oxidation of erythrytol and has the same stereochemical configuration.

Lassar-Cohn and Fringsheim: Zuckerchem. 156 (1925).

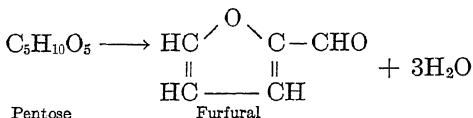
## Ketose

*d*-Erythrulose is metabolized by bacteria. It has the same stereochemical configuration as *d*-erythrose except for the position of the carbonyl group.

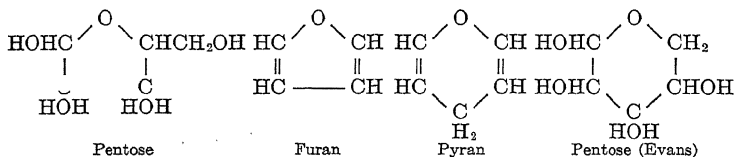
Neuberg: Ber. 35:2627 (1902).

## PENTOSES

Pentoses occur in traces in foods and are distinguished by the fact that pentoses form furfural and other sugars do not.

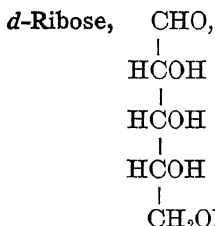


Owing to the fact that the aldehyde (reducing) property of pentoses is weak, a ring structure is postulated. Haworth has attributed a reduced furan ring to some sugars and pyran ring to some others, and the ease with which furfural is produced from pentoses makes the presence of a reduced furan ring a possibility, but Evans found a pyran ring.

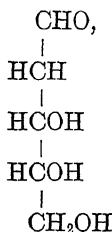


In this furanose formula for pentose the side chain is the sixth carbon, whereas it is generally assumed that the side chain of furfural comes from the first carbon of pentose. The similarity of the rings may be used, however, as an aid to the memory.

## Aldoses



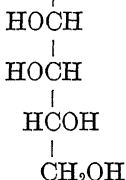
is found in nucleic acid in the nuclei of living cells. It is not agreed what pentoses occur in pentosuria; some suppose it to be ribose.

***d*-Ribodesose (2-deshydroxyribose),**

was shown by Levene to be the carbohydrate of thymonucleic acid (thymine).

Levene, Mikeska, and Mori: J. Biol. Chem. 85:785 (1930).

***d*-Lyxose,** CHO, has been considered as one of the sugars of pentosuria (Zerner).



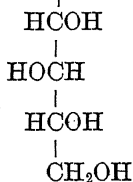
Phelps and Hudson: J. Am. Chem. Soc. 50:2049 (1928).

***l*-Arabinose,** CHO, is formed by the hydrolysis of many gums and woods. Whether arabinose can be utilized by vertebrates is disputed. In the straight-chain formula it has the same stereochemical configuration as arabitol except for the carbonyl group. Arabinose has been found in the urine in pentosuria.



Corley: J. Biol. Chem. 82: 269 (1929).

***l*-Xylose, wood sugar,** CHO, has a straight-chain formula similar to xylitol except for the carbonyl group, and is made from cornstalks by the hydrolysis of the contained xylosan. Xylose is 40% as sweet as cane sugar and would probably be a good sweetener



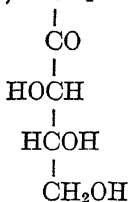
for reducing diets. It is fermented by certain bacteria, yielding equimolar parts of acetic and lactic acids.

Various pentoses undergo this type of fermentation in the gut. The acids produced by this type of fermentation have a laxative effect. Only traces of pentoses occur in nature except in pentosuria.

Corley: J. Biol. Chem. 70:521 (1926).

### Ketose

**Xyloketose**,  $\text{CH}_2\text{OH}$ , in the straight-chain formula is similar to xylitol except for the presence of the carbonyl group. Xyloketose has been found in the urine in pentosuria.



### Methyl Aldose

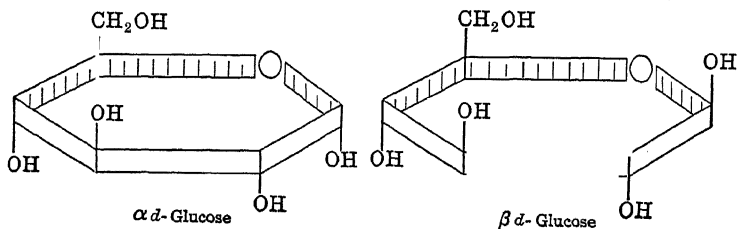
**Rhamnose** occurs in plants and has been said to be found in human urine. It has the configuration of *l*-mannose with the sixth carbon reduced to a methyl group.

Peterson, Fred, and Martin: J. Biol. Chem. 70:309 (1926).

### HEXOSES

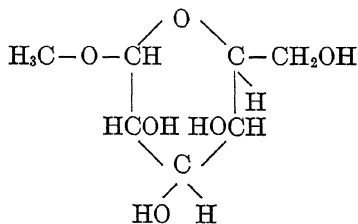
#### Aldoses

***d*-Glucose**, fig. 47, occurring in two isomeric forms,  $\alpha$  glucose and  $\beta$  glucose, is the sugar of the blood.



This is the pyranose formula of Haworth (compared with pyran). The specific rotation is  $+52.6^\circ$ , which is the equilibrium value between  $\alpha$  and  $\beta$  *d*-glucose. On dissolving one or the other of these forms in water, there is mutarotation to equilibrium (which is hastened by alkali).

Glucosides have been made having two forms,  $\alpha$  and  $\beta$ , with different optical rotations:  $\alpha$  glucose may be obtained by hydrolysis of  $\alpha$  methyl glucoside, and  $\beta$  glucose from  $\beta$  methyl glucoside.



Methyl glucoside ( $\beta$  form)

Claude Bernard did the first notable work on the physiology of sugar. He studied the sugar of the blood and showed that after a meal of carbohydrate was eaten, the sugar in the portal vein was increased. Sugar is stored in the liver as glycogen (sugar-former). In a fasting animal the glycogen breaks down into sugar. The liver contains enough glycogen to last about a day. If a great excess of sugar is taken, the excess gets by the liver. This condition is known as alimentary hyperglycemia to distinguish it from hyperglycemia due to other causes. The level for fasting persons is 80–100 mg. glucose per 100 cc. blood as determined by reduction tests. Glutathione may account for about 18 mg. of this figure.

Von Mehring and Minkowski showed that diabetes mellitus is produced by cutting out the pancreas. The blood sugar is higher than normal. Murlin and Kramer prepared effective pancreatic extracts. Banting, Best, Macleod, and Collip were the first to succeed fully in injection of pancreatic extract into men. The extract was called *insulin*.

There are in the pancreas, not connected to the ducts, groups of cells called the islands of Langerhans. These islands secrete insulin. Even if the secreting acini degenerate following ligation of the ducts, diabetes is not developed, because the islands function independently. Insulin is a protein-like substance. At first,

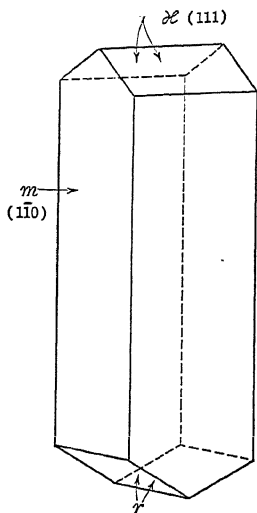


FIG. 47. Crystal or anhydrous *d*-glucose. Industrial and Engineering Chemistry.

difficulty was experienced in preparing it as it is destroyed by pancreatic proteolytic enzymes, which are absent, however, from the fetal pancreas. Collip obtained insulin by cutting the pancreas out of a live animal and immediately placing it in cold alcohol, which stopped the enzyme action. The insulin content of the body is increased after taking sugar (see below).

The blood-sugar level is dependent on the secretion of insulin by the pancreas, as shown by the simple experiment of the glucose-tolerance test, which consists of fasting for 14 or more hours, usually between dinner and the following morning, and then taking 50 to 500 (usually 100) g. of glucose. The blood-sugar immediately rises, and in the diabetic it stays up for hours. In the normal person, however, it rapidly falls even though the glucose is not all absorbed and goes down to normal or below normal within a couple of hours. On taking a second dose of glucose the diabetic has a second rise equal to the first, but the normal person shows a very insignificant or no rise. This phenomenon is interpreted to show that the absorption of glucose stimulates the pancreas to secrete insulin into the blood in a normal person. Macallum has isolated a hormone that causes the pancreas to secrete insulin. By glucose-tolerance tests partial diabetes may be quantitatively estimated.

Although a fast of about 14 hours is the standard, it has been shown that the longer the fast the slower the pancreas is to respond. Instead of a fast, a carbohydrate-free diet or one which supplies only small amounts of glucose-forming substances may be used. A fat diet supplies only glycerol as a glucose-forming substance, but 58% of protein is glucose-forming in the body. As these diets are continued for days, weeks, months, and years, the pancreas becomes more and more inactive to glucose-tolerance tests; in fact, after a year or more on a "carbohydrate-free" diet, a normal person becomes temporarily partially diabetic on returning to a carbohydrate diet but recovers spontaneously, owing possibly to the production of Macallum's hormone.

With the rise of blood-sugar on giving glucose after a fast, the average person shows sugar in the urine when the blood-sugar reaches 170 mg. per 100 cc., but in some persons it may go above 200 without glycosuria. Some persons show sugar in the urine with a normal blood-sugar. They have been called renal diabetics, but it would be more conservative to call them mild diabetics.

The renal threshold varies greatly in normal individuals as well as in diabetics, and therefore a sugar-tolerance test with determination on the urine only is not of much value. Some normal people show sugar in the urine with 150 g. of glucose after a 14-hour fast; others do not show sugar in the urine after 500 g. of glucose following a 14-hour fast.

On injecting insulin into a normal person or an overdose into a diabetic, the blood-sugar falls below normal and may continue to fall for 4 hours or more if no carbohydrate is administered. When it reaches a value which is estimated as 30-50 mg. per 100 cc. blood, depending somewhat on whether it is venous or capillary blood and on the method of determination, hyperinsulinism is observed. This is usually called insulin-shock, although it should not be implied that it is similar to surgical shock. Deaths in man have been rarely observed although it is possible to kill any small mammal with insulin. The fact that no one has produced death in the cow with insulin may be due to the difficulty in obtaining enough insulin. The most customary symptom in man is loss of consciousness, although twitching and weakness are observed in cases that do not reach the unconscious stage.

Hyperinsulinism is often diagnosed as epilepsy unless the case history indicates to the physician its true nature. Some persons have an abnormal pancreas which produces too much insulin, and have to carry sugar in their pockets to save them from collapse. A number of these people have been carried to hospitals unconscious and the disease diagnosed as epilepsy. In one case the patient remained unconscious 3 days under this diagnosis.

Certain symptoms observed in animals have been considered insulin-shock symptoms. It was shown by Greenwald that after a dog is dehydrated and then given insulin, the shock symptoms are absent, although the lethal dose is not raised, and he interprets the effect of insulin as causing water to leave the blood and rush to the brain, the shock symptoms being due to hydration of the brain. This would bring insulin-shock in line with epilepsy because dehydration, as shown by McQuarrie, prevents epileptic attacks. The difference would be in the cause of brain hydration, which is hyperinsulinism in one case and an unknown factor in the other.

A graph is shown in fig. 20, p. 48, on supposedly normal persons, giving the blood-sugar and blood-volume after a 14-hour fast, followed by a diet of glucose and water. The blood-sugar

and blood-volume are referred to the initial values as 100. In one case insulin was given and the blood-sugar dropped in 4 hours below 50 mg. per 100 cc. No water was given for 8 hours and the blood-volume dropped to below 90. On the other hand, when no insulin was given but sugar and water were drunk (50 g. of glucose every hour in 200 cc. of water except for the first dose, which was given in a liter of water) the blood-sugar rose rapidly to above 180, then fell to a new level of about 135 in 2 or 3 hours and slowly fell to the end of the seventh hour with a more rapid fall to the end of the eighth hour.

The blood-volume rose steadily for 7 hours up to nearly 140% and then began to fall.

Such huge increases in blood-volume seem incredible, and it is possible that these figures are unusual in either the types of individuals selected or in the method of estimating changes in blood-volume, which was by the hemoglobin-content. The spleen is known to be a reservoir of erythrocytes and accumulation of them in the spleen would be interpreted as a rise in blood-volume. Whatever errors might have been introduced, it seems evident that there was a fall in blood-volume on giving insulin and a rise in blood-volume on giving glucose and water. The same quantity of water, without glucose, is very rapidly excreted in the urine and shows only a transitory effect on the blood-volume.

The mechanism of insulin action is obscure and only vague speculations may be given. It has been reported by several observers that the blood of normal people contains  $\gamma$ -glucose and that of diabetics  $\alpha\beta$  glucose.  $\gamma$ -Glucose may be produced in solution by the hydrolysis of  $\gamma$ -glucoside but it rapidly changes over into the  $\alpha\beta$  form. There has been much speculation on the structure of glucose. The formula given above is that of Haworth, who describes the  $\alpha\beta$  glucose as glucopyranose having a ring of 5 carbon atoms and 1 oxygen atom. He believes the  $\gamma$ -glucose is glucofuranose having a ring of 4 carbon atoms and 1 oxygen atom as shown in sucrose, below.

The basis of the belief that normal blood contains  $\gamma$ -glucose is the simultaneous determination of its reducing power and optical rotation, but it must be remembered that blood contains other reducing substances besides glucose, as well as other optically active substances.

The immediate effect of insulin is to lower the blood-sugar, but

the mechanism of the lowering is not clear. Insulin enables the diabetic to burn sugar, as otherwise he cannot utilize it and 100% passes out in the urine; but the lowering of the blood-sugar is not to be attributed entirely to the burning of sugar. Evidently, insulin must assist in the storage of sugar. Sugar is stored as such in various tissues, such as the muscles (which are the most voluminous tissues) and the skin, and it is partly polymerized and stored as glycogen and possibly also in the form of glucose phosphate.

Armstrong: Simple Carbohydrates and Glucosides, Longmans, Green & Co., New York (1924).

Banting: Can. Med. Assoc. J. (1922).

Evans: Chem. Rev. 6:281 (1929).

Folin: J. Biol. Chem. 70:405 (1926); 77:421 (1928); 83:115 (1929).

Haworth: The Constitution of Sugars, London (1929).

Hudson: J. Am. Chem. Soc. 32:338 (1910).

Levene: Chem. Rev. 5:1 (1928).

Macleod: Physiol. Rev. 1:208 (1921); Stammers: *Ibid.* 6:630 (1926).

In 1923, Collip in Canada, and Winter and Smith in England, independently isolated from yeast a substance acting like insulin which they called *glucokinin* ("gluco" = sugar, and "kinema" = motion). All plants investigated have yielded glucokinin, but they also yield other substances that raise the blood-sugar so that the glucokinin has to be purified. Collip kept a depancreatized dog alive for 66 days on an extract from onions containing glucokinin.

W. H. Eyster discovered a disease of Indian corn plants which he calls diabetes. Sugar accumulates in the leaves until the latter are ruptured. It seems possible that glucokinin is necessary for sugar metabolism in the plant. In Indian corn, sugar is stored as starch, but in yeast, which is a good source of glucokinin, glycogen is stored.

Collip: J. Biol. Chem. 57:65 (1923).

Determinations of the amount of glucose formed in the body from any particular foodstuff were made by Lusk. His method consisted in making an experimental animal diabetic (either by depancreatization or by administration of phlorizin every 8 hours) and then administering the food. From urinary sugar, nitrogen-excretion, and gaseous metabolism the amount of sugar produced from the food may be calculated. Below are some of the com-

pounds which may be transformed into glucose in the body of a diabetic:

<i>Starch</i>		} All these produce glucose 100% in the body	
Glycol aldehyde	$\text{CH}_2\text{OHCHO}$ (a biose)		
Glycerol	$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}_2\text{OH}$		
Lactic acid	$\text{CH}_3\text{CHOHCOOH}$		
Propyl alcohol	$\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$		
Methyl glyoxal	$\text{CH}_3\text{COCHO}$		
Fructose	$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{CO}\cdot\text{CH}_2\text{OH}$		
		<i>Observed</i>	<i>Theoretical</i>
Glycine	$\text{CH}_2\text{NH}_2\text{COOH}$	13.43	16.00 g.
Alanine	$\text{CH}_3\text{CHNH}_2\text{COOH}$	18.77	20.22
Aspartic acid	$\text{COOHCH}_2\text{CHNH}_2\text{COOH}$	12.42	13.52
Glutamic acid	$\text{COOHCH}_2\text{CH}_2\text{CHNH}_2\text{COOH}$	13.31	12.24

The ratio of glucose to nitrogen in the urine may be called the G/N ratio (also called D/N ratio) and is 3.625 for total diabetics metabolizing protein only. The fate of 100 g. protein is shown graphically in the accompanying figure.

58% glucose	23% burned
19% $\text{NH}_3$	

Lusk: Science of Nutrition, Saunders, Philadelphia, fourth edition (1928).

In the feeding of a diabetic, a quantitative relation between the glucose equivalent of the food eaten and insulin intake is desirable. Early samples of insulin were not well standardized, and patients became unconscious at times and were unable to find their way back to the hospital. Therefore, sufficient carbohydrate should be given with a certain dose of insulin.

On the other hand, too large an intake of food makes the diabetic feel badly. This feeling may be due partly to the loss of water from the body, since the excretion of sugar in the urine is accompanied by the excretion of water. Over-eating by a diabetic in the absence of sufficient insulin may result in diabetic coma. The mechanism of this condition is not clear, but certain factors have been worked out. One is dehydration, and therefore physiological salt solution is injected in such cases; the second is ketosis.

Ketosis may not be entirely due to a lowering of the insulin intake, but with a given intake of insulin it may be due to an increase in the basal metabolic rate due to over-eating, and since extra energy cannot be derived from sugar an unusually large amount of fat is burned and therefore there is ketosis. Along with the ketosis there is an increased loss of sodium from the blood due to the excretion of sodium- $\beta$ -hydroxybutyrate. With the loss of sodium there is a lowering of the excitability of the nerves. Perhaps the symptoms are due to combined dehydration and loss of sodium. The pH of the blood is unstable, and uncompensated acidosis may be present, with additional depression of irritability.

It is safe for the diabetic to weigh his food and calculate the available carbohydrate in it. A list of foods *low in glucose equivalent* is as follows:

#### VEGETABLES (cooked except where marked raw)

1% glucose equivalent: French artichoke, scarlet runner bean, cabbage, cauliflower, celery (raw), egg plant, lettuce (raw), spinach, rhubarb.

2%: Jerusalem artichoke, asparagus, cucumber (raw), tomato.

3%: rutabagas, Brussels sprouts, pumpkin.

4%: turnips.

5%: onions; 6%: carrots; 8%: beets; 9%: parsnips; 12%: peas; 16%: lima beans; 17%: haricot beans; 19%: potatoes.

Those that contain only 1 and 2% carbohydrate are leafy vegetables, and the parts that are eaten are exposed to the sunlight; those that contain 3 to 19% carbohydrate are storage organs.

#### FRUITS (raw)

1%: glucose equivalent: cranberries.

2%: lemon juice.

3%: black currants, loganberries, watermelon, raspberries.

4%: red currants, strawberries; 5%: blackberries, damson plums, apricots, gooseberries; 6%: cherries, grapefruit, green gage plums, oranges; 7%: nectarines, peaches, pears; 8%: apples; 9%: persimmons, plums; 10%: pineapple, grapes; 14%: figs; 19%: banana.

#### DRIED STEWED FRUIT

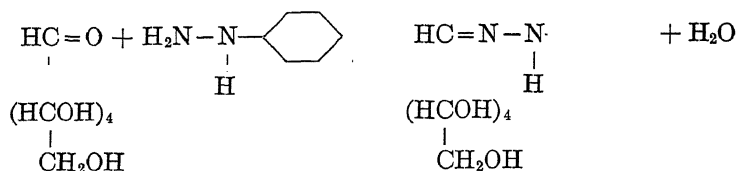
9%: apricots; 14%: prunes; 19%: figs.

#### NUTS

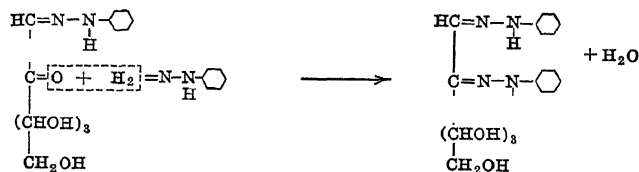
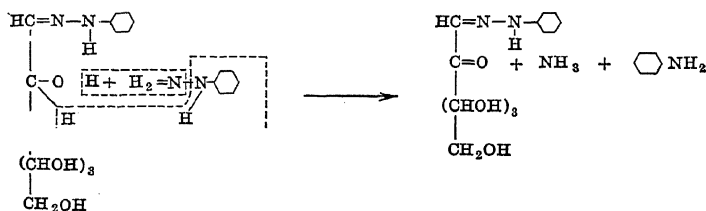
3%: Brazil nuts; 5%: cocoanuts, walnuts; 7%: almonds, hazel nuts; 11%: peanuts; 29%: chestnuts.

McCance and Lawrence: Med. Research Council, Sp. rpt. ser. 135, London (1929).

**Osazones.** If phenylhydrazine is added to a glucose solution, glucose phenylhydrazone is formed as an unstable intermediate owing to splitting out of water:



By further action of phenylhydrazine, there is oxidation of the next (second) carbon atom and formation of aniline. Union with another molecule of phenylhydrazine then forms phenylglucosazone. So it takes 3 molecules of phenylhydrazine to change glucose to osazone.



Phenyl glucosazone

Various sugars are determined qualitatively by the crystalline form and the melting-points of their osazones. Glucose, fructose, and mannose form the same osazone.

Knecht and Hibbert: J. Chem. Soc. 125:2009 (1924).

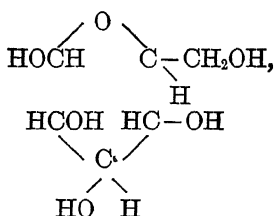
**Glucose Phosphate, Lactacidogen.** Meigs in 1912 observed the formation of lactic acid and phosphoric acid in equimolecular proportions in excised muscle. The substance from which they were formed was called lactacidogen and has been studied by a number

of workers. It has also been demonstrated that there is a relation between the phosphate in the blood and the blood-sugar. Two substances have been studied, glucose monophosphoric acid and glucose diphosphoric acid. An enzyme (phosphatase) splits them into glucose and phosphoric acid, and glucose may be split further into lactic acid. Harden isolated these substances from yeast-glucose fermentations. Glucose diphosphoric acid was originally designated as "lactacidogen."

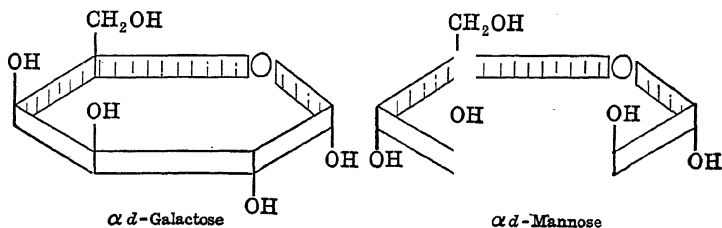
Emden and Jost: Z. physiol. Chem. 179:24 (1928).

Euler and Myrbäck: Ann. 464:56 (1928).

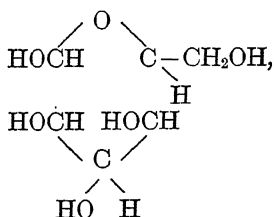
***d*-Galactose,**



has 95% of the reducing power of glucose and one-third the sweetness of cane sugar. It is utilized, especially in growing animals, in forming the galactosides of brain and nerves. It is absorbed more slowly than glucose. If an Eck-fistula (connection between portal vein and inferior vena cava) is made in a dog and galactose is fed, it is excreted in the urine. The normal galactose tolerance is 30 g. for men and 40 g. for women. An excretion of more than 3 g. in the urine on eating these respective amounts is considered low tolerance. It is stored as glycogen.



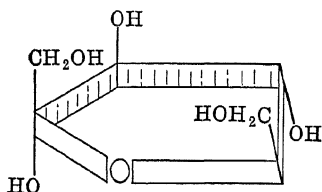
Cori: J. Biol. Chem., 70:577 (1926).

*d*-Mannose,

occurs free in only a few plants. It is absorbed more slowly than galactose but is changed into glucose in the body.

Cassidy, Dworkin, and Finney: Am. J. Physiol. 77:211 (1926).

## Ketoses

*d*-Fructose,

The *d* does not refer to its optical rotation (which is levo "levulose") but to the fact that the arrangement of the last four carbon atoms is the same as that of *d*-glucose. Glucose and fructose can be distinguished by their optical rotations but not by their osazones. Fructose is 173% as sweet as sucrose.

Von Mehring and Minkowski claimed that if fructose (which is a keto-hexose related to glucose in its steric structure) is given to diabetics, it is stored as glycogen in the liver, whereas glucose is not stored. Fructose is so expensive that this question has not been absolutely settled. Fructose is obtained by the hydrolysis of inulin, a polysaccharide in Jerusalem artichokes. When cane sugar is hydrolyzed, equal quantities of fructose and glucose are formed and these may be separated. At present fructose from cane sugar is cheaper than fructose from inulin but it is not free from glucose. Since glucose may be formed in the body from protein as well as from fructose it would seem futile to feed fructose absolutely free from glucose to a diabetic when it is so expensive.

When fructose is injected into the blood, some may pass into



might take place. W. L. Evans has studied these changes along with oxidations, and they are summarized in fig. 48.

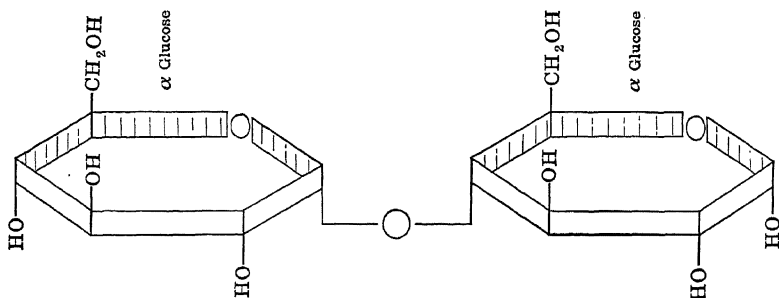
Evans: Chem. Rev. 6:281 (1929).

Shaffer: Physiol. Rev. 3:394 (1923).

### DISACCHARIDE SERIES

Disaccharides are formed by glucoside union between 2 monosaccharide molecules. Methyl glucoside has lost its reducing power as the methyl group is attached to the carbon atom where oxidation would take place. Some disaccharides are reducing and some not reducing, depending on the mode of linkage. Pentose disaccharides are not utilizable in the body.

**Maltose**, glucose 4, $\alpha$ -glucoside, is produced by hydrolysis of starch by the enzyme diastase, particularly in the germination of



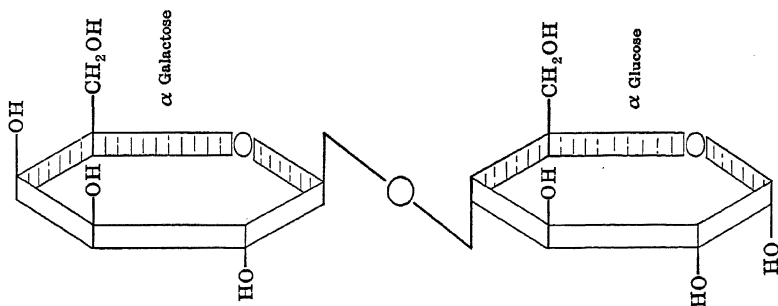
barley seedlings, which are known as malt. It reduces Fehling's solution. It is 32.5% as sweet as sucrose.

Mitchell: Am. J. Physiol. 79:537 (1927).

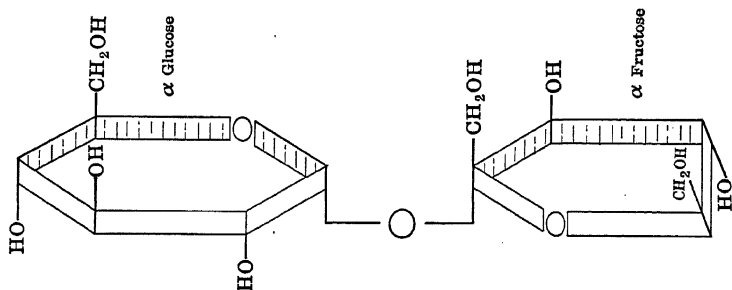
**Lactose**, glucose 4, $\beta$ -galactoside, occurs to the extent of about 4.5% in cow's milk and 7% in human milk. Very few yeasts contain lactase but it is found in Kephir grains. It is split in the gut into glucose and galactose, but this splitting is so slow that some of it is attacked by bacteria in the meantime, this fermentation producing fatty acids. These acids lower the *pH* of the gut. It is synthesized in the mammary glands. The normal lactose tolerance is 10 g. for both male and female but during pregnancy it increases in women to 20-30 g. The significance of lactose in the milk probably lies in the fact that it is hydrolyzed by lactase in the small intestine to glucose and galactose and the galactose is absorbed, and although most of it is changed to glyco-

gen in the liver, some of it may be used to synthesize galactosides in the nervous tissue where they form part of the myelin sheath of nerves. It is 16% as sweet as sucrose.

Greenwald and Gross: J. Biol. Chem. 82:505 (1929).



Sucrose, cane sugar, beet sugar,  $\alpha$ -glucopyranosido  $\beta$ -fructofuranoside, is the most abundant sugar in nature, occurring not only in the sugar cane and sugar beet but in many vegetables in which one would not suspect sugar, such as onions, and also in all fruits. It does not reduce Fehling's solution, owing to the fact that the union between the two sugar molecules is on the



carbon atoms which contain the carbonyl in the straight-chain formula. It therefore does not exhibit mutarotation or form compounds with phenylhydrazine. It will stand heating in alkaline solutions up to  $130^\circ$  without appreciable decomposition. The free hydroxyl groups, however, enable it to form saccharates with various metals.

It is hydrolyzed by the enzyme invertase and also by the  $\text{HCl}$  of the stomach. Its specific optical rotation is  $+66.67^\circ$  at  $20^\circ$ .

On hydrolysis the optical rotation is the algebraic sum of those of glucose, which is  $+52^\circ$ , and of fructose, which is  $-92.09^\circ$ . A negative rotation results, the change being called inversion and the product invert sugar. Honey bees suck up the nectar of flowers, which is largely a solution of sucrose, and invert it in their alimentary canals and then regurgitate it into the honey comb, where it is inspissated by the fanning action of their wings to form honey. Honey is invert sugar and is useful in making candy and ice cream, as it does not easily crystallize. Sucrose is partially inverted by boiling with vinegar. In making chocolate-coated cream candy, invertase is mixed with the fondant and hydrolyzes the sucrose after the candy is dipped.

Sucrose is sweeter than most sugars except fructose. It becomes sweeter on inversion, on account of the fructose formation.

Sucrose gives a blue-green color with diazo-uracil (Raybin).

Haworth and Hirst: J. Chem. Soc. 1858 (1926).

Raybin: J. Am. Chem. Soc. 55:2603 (1933).

### POLYSACCHARIDE SERIES

Polysaccharides are condensation products of monosaccharides and may be all of one monosaccharide or they may be mixed. When formed of pentoses, they are known as pentosans and are gummy or mucilaginous in nature.

Among the polysaccharides might be included the so-called aldobionic acids from type A Friedlander *Bacillus*, type 3 *Pneumococcus*, and *B. tuberculosis*, since hydrolysis has yielded monosaccharides and disaccharides. It is thought by some workers that these carbohydrates are specific antigens.

Gough: The specific carbohydrate of tubercle bacillus, Biochem. J. 26:248 (1932).

#### PENTOSANS

##### Pentosans (McCance)

	Per cent
Pears (raw).....	2.25
Cabbage (boiled).....	1.25
Cauliflower (boiled).....	1.25
Parsnips (boiled).....	2.38
Dried apricots (stewed).....	1.34
Young lima beans (butter beans, boiled).....	3.29
Dried bran.....	61.5
Walnuts (kernels).....	2.05
Almonds (kernels).....	5.48
Peanuts (kernels).....	3.8

Pentosans occur in all natural vegetable foods. The normal daily intake does not exceed 12 g., of which not more than 8 g. is fermented to fatty acids. Owing to the stimulating action of these acids on the intestinal secretions the bulk of the feces is increased. Too heavy doses of pentosans in the diet (as in beans and nuts) cause noticeable fermentation, and a prolonged indulgence may cause fermentative dyspepsia. The spasm of the gut after eating too many green apples or the chronic spasm in fermentative dyspepsia may be due to the fatty acids produced by fermentation.

**Arabinose** is polymerized to **araban** (gum arabic), **xylose** to **xylan**, and **ramnose** to **ramnan**. No pure samples of these pentosans have been found in nature. The presence of polysaccharides in cactus and other desert plants causes them to hold water in a dry climate.

Link: J. Am. Chem. Soc. 51:2506 (1929).

**Agar-agar** (Ceylonese) is obtained from red seaweed by the Japanese and sold under the name of "kanten." The water is removed from it by freezing. It is a calcium compound of a mixed polysaccharide yielding galactose and pentoses on hydrolysis.

Agar liquefies when the calcium is broken off by treatment with acid or electrodialysis. This fact alone refutes the contentions of those who believe agar gel passes through the alimentary tract unchanged, although it may gel again after being liquefied in the stomach. It is broken down by certain marine and soil bacteria, and has not all been recovered in the feces of rabbits. Since many bacteria do not attack it, it is used in bacteriology for preparing gel media.

Myake: J. Col. Agr. Japan 4: (No. 8).

**Alginic acid** is a polysaccharide forming 20-40% of the dry weight of the brown seaweed *Laminaria*, from which it is extracted with 0.5%  $\text{Na}_2\text{CO}_3$ , filtered and precipitated by adding acetic acid. Oshima found alginase in sea urchins and abalone "liver." Another brown seaweed, *Fucus*, is eaten in Scotland under the name of dulse. Its polysaccharide is said to be hydrolyzed in the gut.

Oshima: Bull. Agr. Chem. Soc. Japan 7:332 (1931).

## HEXOSANS

**Glycogen** is a hexosan storage product in living cells of our bodies. It may occur in all cells but the chief stores are in the liver, which may contain 17% (about 300 g.), muscle, which may contain about 0.5% (or 300 g.), and the skin.

Adrenaline causes hydrolysis of muscle glycogen (Cori) and seems to have something to do with glycogen-hydrolysis in the liver, but the relation is not clear. On its injection, blood-sugar and respiratory quotient rise.

Insulin increases the storage of glycogen. Recent work distinguishes between the storage in the muscles and in the liver, and indicates a complicated mechanism. All tissues of the body have the power to split glucose into *lactic acid*, and this may be transported to and *stored as glycogen in the liver*. The livers of animals that have been diabetic for a long time show very little glycogen even though fed carbohydrate. Giving large doses of insulin to fasting rats sometimes lowers the glycogen in the liver, supposedly by increasing the burning of glucose. By prolonged baths in ice water or by strychnine convulsions, the liver may be freed of glycogen (Lusk).

Meyerhof claimed that during muscular contraction glycogen is changed to lactic acid, and during the resting phase,  $\frac{2}{4}$  or  $\frac{1}{3}$  of the lactic acid is re-formed into glycogen. If lactic acid escapes into the blood some is changed to glycogen in the liver; otherwise glycogen cannot pass from muscle to liver. Mann developed the technique of complete removal of the liver, in which blood-sugar rapidly falls to the convulsive level, although the muscles contain much glycogen. This is of physiological advantage since the muscles need the glycogen for muscular work, but this cannot be explained from the standpoint of chemical equilibria.

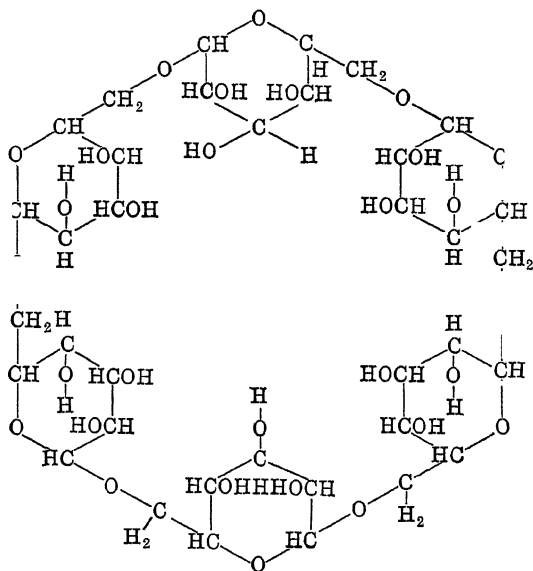
Bollman, Mann, and Magath: *Am. J. Physiol.* 74:238 (1925).

Cori: *Harvey Lectures*, 1927-8:76 (1929); *Physiol. Rev.* 11:143 (1931).

Cori and Cori: *J. Biol. Chem.* 79:309 (1928); 81:389 (1929).

Meyerhof and Lohmann: *Biochem. Z.* 177:421 (1926).

Starch.



The molecular weight and structural formula of starch are unknown, but it yields maltose and glucose on hydrolysis. The above formula is similar to one suggested by Irvine and accounts for its colloidal properties as distinguished from those of cellulose.

Starch is the chief hexosan storage product of plants, since cellulose may be considered a skeletal element. Starch does not dissolve in cold water but if boiled with water the scales or concentric layers, of which the starch grain is made, break. It is then seen to be formed of two different substances in alternate layers, one of which is more soluble than the other but the whole forming a more or less colloidal solution. The center of the starch grain appears to contain glucose phosphate.

By a very mild hydrolysis it is claimed that soluble starch may be formed which disperses in cold water, but many samples of so-called soluble starch are really dextrin.

In the digestion of starch in the mouth, dextrin is first formed, as may be shown by the iodine test. Iodine gives a blue color to starch. In order to produce the blue color, both iodine and iodide are necessary, but usually in starch solutions enough reducing

substance is present to reduce a trace of the iodine to iodide and develop the blue, or iodide may be added, as in Lugol's solution (iodine dissolved in KI solution). Iodine gives a pink color to dextrin. With only a very faint trace of iodine, starch gives a pink color, but this may be distinguished from dextrin by adding more iodine. On further digestion the color reaction is lost before all the polysaccharide is broken down and it is, therefore, named achroodextrin. During digestion maltose is produced. The enzyme concerned is *diastase*, but if the splitting goes to glucose, maltase is said to be present. Both of these enzymes are included under the name of ptyalin in the saliva.

Alsberg: Ind. Eng. Chem. 18:190 (1926).

### Cellulose.

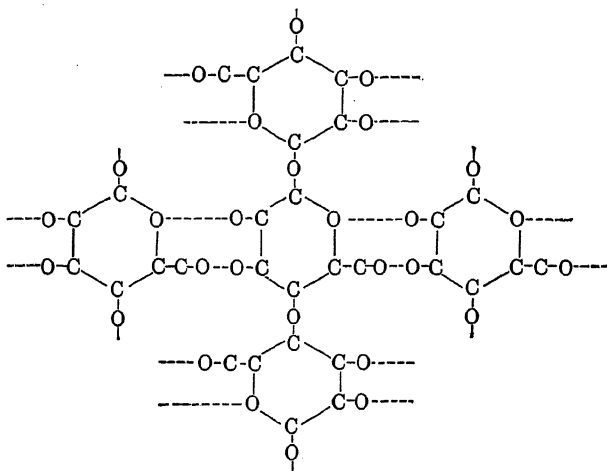


Diagram (omitting hydrogen atoms) to show glucoside union of glucose molecules in chains, the chains being united by residual valences (dotted lines).

Cellulose is the chief constituent of vegetable fibers as well as of the cell walls of most plant cells. Autoclaving it with acid produces glucose. The glucose molecules are arranged in long chains parallel to the direction of the fiber, the chains being united by residual valences.

Cellulose may be dissolved and then solidified again in the form of threads by forcing the solution through fine openings into dry

air or a coagulating agent and thus is formed into artificial silk. In artificial silk the chains are not all parallel to the fibers and it has not the strength of the natural fibers. Besides being formed into threads, as in rayon clothing, it is made into sheets called cellophane and into sausage casings known as Visking. These Visking sausage casings are very useful in purifying chemical substances by dialysis. The casing may be coiled in a solution of the substance and water run through it from a tap, the water carrying away the impurities which pass into the casing. Water-proofed cellophane cannot be used for dialysis.

	Per cent Cellu-		Per cent Cellu- lose
Corn meal.....	1.0	Onions, fresh.....	0.8
Rolled oats.....	1.3	Parsnips.....	2.5
Rice.....	0.2	Peas, green.....	1.7
Wheat flour.....	0.2	Potatoes, raw.....	0.4
Asparagus, fresh	0.8	Spinach, fresh.....	0.9
Beans, dried....	4.4	Tomatoes, fresh.....	0.6
Beets, fresh.....	0.9	Apples.....	1.2
Cabbage.....	1.1	Bananas.....	1.0
Carrots, fresh...	1.1	Blackberries, as purchased	2.5
Celery.....	1.0	Cherries.....	0.2
Cucumbers.....	0.7	Cranberries, as purchased	1.5
Lettuce.....	0.7		

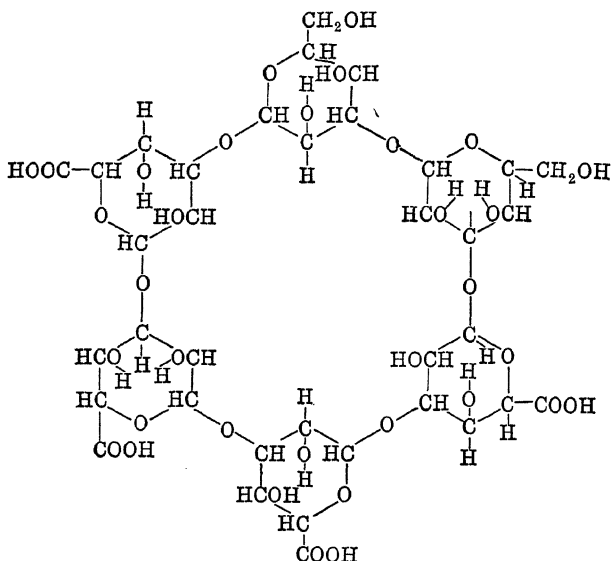
When cellulose is treated with nitric acid, nitrocellulose is formed, which is known as guncotton and is explosive. Mixed with camphor it forms celluloid, which is used for X-ray films, the storage of which is accompanied by great hazards. In order to prevent these hazards, acetylated cellulose is substituted for the nitrated cellulose.

Khouvine isolated a bacillus from the gut requiring cellulose.

Cellulose combines with phenolic compounds or unsaturated rings to form lignocellulose. Sodium sulfite solution dissolves the lignin, so that paper pulp is cellulose.

Gortner: Outlines of Biochemistry, Wiley, New York.

**Pectic acid** differs from starch in that part of the glucose is oxidized to glycuronic acid, which makes it more soluble. The following formula has been proposed to account for its colloidal properties:



Some pectic acids yield some pentose in addition to glycuronic acid on hydrolysis.

Wichmann and Chernoff: J. Assoc. Official Agr. Chem. 8:129 (1924).

**Pectin** is the calcium salt of pectic acid. If the calcium is removed, pectin is more soluble. It is the pectin that causes fruit jellies to gel; but if the acidity is so great that the calcium is removed, the jelly will not gel.

Ehrlich and Summerfield: J. Am. Chem. Soc. 49: Proc. 37 (1927).

Besides these polysaccharides some less well-known vegetable mucilages have been considered to be hexosans. They include:

**Galactan** is said to occur in the cell walls of plants. Agar, already referred to, is a mixed galactan-pentosan.

Beirry: Biochem. Z. 40:370 (1911).

**Mannan** is widely distributed. Marie Rose claims that salep mannan is 96% digestible by humans, but this digestion is probably by bacteria in the gut. It can be used to make a gel.

Rose: J. Biol. Chem. 42:159 (1920).

**Inulin** is a fructosan occurring in tubers of Jerusalem artichoke, dahlia, and chicory. There is no digestive enzyme secreted by

humans to digest it, but it is utilized if less is taken than will cause diarrhea; the products of bacterial action in the intestine are acetic, lactic, and other fatty acids.

Sachs: Biochem. Z. 117:227 (1921).

**Dextran** is secreted by bacteria causing ropiness in wine and so-called frog-spawn in sugar fermentation.

Delage: Chim. Ind. 14:592 Sp. No. (Sept. 1925), 4th. Cong. Chim. Ind.

**Available Carbohydrate Content of Foods.** Disaccharides and some polysaccharides and glucosides yield monosaccharides on digestion. Glucose, fructose, and sucrose are fermented very little in the gut, maltose and lactose being fermented more because they remain longer in the gut, being hydrolyzed and absorbed more slowly.

With the exception of glycogen and cooked starch, the polysaccharides (cellulose, agar, inulin, pentosans, and various gums and mucilages) are called roughage. When raw food is eaten it may contain enzymes to hydrolyze roughage. They are fermentable and yield acetic, lactic, propionic, and butyric acids which stimulate the gut to yield mucus, detritus, and other products of metabolism, "Stoffwechselsprodukte" (Rubner).

There are many bacteria in the colon, but as many of them are dead and sufficient water is lacking not much fermentation takes place there. The colon is an organ for removing water from the intestinal contents. The less water there is, the less fermentation occurs.

Fermentation occurs mainly in the small intestine. The more rapid the fermentation the more rapid the transportation of food. The condition in which much fermentation goes on in the colon is called fermentative dyspepsia.

Bran acts mainly as roughage. Sauerkraut contains lactic acid due to fermentation during preparation, and pickles contain acetic acid due to fermentation of malt or cider in the preparation of the vinegar. More of these acids are produced if sauerkraut and pickles are eaten, as they are fermented in the gut.

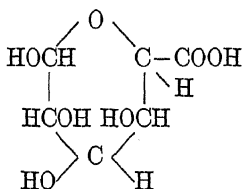
## GLYCOSIDE SERIES

A glycoside is composed of one or more sugar molecules attached by glucoside union (as in methyl glucoside mentioned in consideration of the structure of  $\alpha\beta$  glucose) to an "aglucone" or non-

sugar group. There are a large number of glycosides (hexosides and pentosides) synthesized in plants. Their significance is not always known. It is possible that they protect the plants from being eaten by animals since many of them are of an intensely bitter taste and are toxic. On the other hand, one might reason, from analogy with glycosides formed in the animal body, that they represent detoxication compounds, that is, compounds whose function is to lessen the toxicity of other substances as shown in the next paragraphs.

**Uronic Acids.** Many drugs after absorption into the body form glycosides with glucose (and possibly other sugars). These seem to be quite stable substances. The part of the glucose in the pyranose ring resists oxidation, but the sixth carbon which is in a side chain is oxidized to a carboxyl group and thus glucose is transformed into glucuronic acid.

Glucuronic acid,



has been produced by oxidation of glucose by  $\text{H}_2\text{O}_2$  (Jolles) but, according to Fischer and Piloty, is never formed except when the glucose is in glu-

coside union. These glucosides of glucuronic acid, known as glucuronates, are excreted in the urine as is glucuronic acid injected intravenously. They may be formed in the intestinal wall and the liver because it is stated that they may be absent in hepatic disease.

Jolles: *Biochem. Z.* 34:242 (1911).

**Glucuronates.** Among the drugs which are excreted as glucuronates are camphor, borneol, menthol, phenetiden, acetanilide, resorcinol, chloral hydrate, chynsol, sandal-wood oil, naphthol, pyramidon, antipyrine, turpentine, chloroform, prussic acid, morphine, butyl-chloral hydrate, arsenic, curare, strychnine, phloroglucinol, phenol, cresol, isopropyl alcohol, thymol, naphthalene, indole, skatole, benzoic acid, hydroxyquinoline, and *o*-nitrotoluene.

The primary and secondary alcohols are conjugated as such, whereas aldehydes and ketones undergo a reduction to the alcohols before conjugation.

Besides their occurrence in the animal organism, glycosides of glucuronic acid occur in plants; thus the dyestuff, Indian yellow, forms a glucuronate known as **euxanthic acid**.

Ehrlich and Rehorst: Ber. (B) 62:628 (1929).

Quick and Kahn: J. Bact. 18:133 (1929).

**Heparin** is supposed to contain glycuronic acid in its constitution and may be classed with the above substances. It contains no sulfur, phosphorus, or nitrogen. Two and one-half milligrams per 100 cc. of blood prevents coagulation. In man 100 mg. triples the coagulation time, which returns to normal in about 2 hours. Its formula is  $C_{15}H_{32}O_{17} \cdot 6H_2O$ ,  $(\alpha)_D + 41.60^\circ$ , and it decomposes at  $250^\circ$ , is hygroscopic, gives the Molisch reaction but does not reduce until hydrolyzed with acid. It is a monobasic acid  $K_a = 2 \times 10^{-4}$ .

Howell: Am. J. Physiol. 47:328 (1918); 71:553 (1925); 77:680 (1926).

Schmitz and Fischer: Z. physiol. Chem. 216:264, 274 (1933).

**Phenolic Glycosides.** There are so many glycosides of glucose and some other sugars in plants that space does not allow us to consider them all. Many of them have been used in medicine. They occur in preparations known as bitters. Some of them (saponins from soap bark) are used in washing, since they lower the surface tension of water.

**Arbutin**, hydroquinone glucoside, is a diuretic and bacterial differentiator.

Gosio: Ann. d'Igiene, 27:213 (1917).

**Phlorizin**, phloretin-glucoside, has been very useful in studying sugar metabolism. V. Meering showed that phlorizin rendered animals glycosuric. The bark (especially of roots) of apple, pear, and plum trees contain it but also an enzyme, phloridase, which hydrolyzes it. The intravenous administration of 5 mg. of phlorizin gives rise to glycosuria, which lasts nearly an hour. In producing diabetes in dogs 1 g. in oil is usually given subcutaneously once every day (Lusk). The details of the action of phlorizin are in dispute, but it seems probable that it is very similar to true diabetes but that insufficient doses cause a mild diabetes. It is said to retard glucose absorption.

Lusk: Science of Nutrition, fourth edition, Saunders, Philadelphia (1928).

Ware: Analyst, 50:384 (1925).

Among phenolic glycosides found in plants are **coniferin**, glucoside of *m*-methoxy-*p*-hydroxycinnamyl alcohol; **salicin**, glucoside of saligenin; **populin**, glucoside of saligenin and benzoic acid; **gaultherin**, glucoside of methyl salicylate.

Kraemer: Science of Applied Pharmacognosy, 587, John Wiley, New York (1928).

**Cyanophoric glycosides** include **prunasin**, glucoside of *d*-mandelonitrile; **amygdalin**, a double glucoside of the same substance; **prulaurasin**, which is racemic prunasin; **dhurrin**, glucoside of *p*-hydroxymandelonitrile; and **linamarin**, glucoside of acetone cyanhydrin.

Armstrong: The Simple Carbohydrates and Glucosides, Longmans, London (1924).

**Peristaltin** from the bark of cascara sagrada is used as a cathartic. **Esculin**, glucoside of esculetin, is said to absorb ultra-violet rays and slowly give them off and has been used as a protective against sunburn (Freund). The lethal dose is 4 g. **Fraxin** is a glucoside of fraxetin.

Wood: U. S. Dispensatory, twenty-first edition, :1186, Lippincott, Philadelphia (1918).

**Anthocyan Glycosides.** **Ruberythric acid**, glucoside of alizarin; **apiin**, glucoside of apigenin; **quercitrin**, glucoside of quercetin; **rutin**, glucoside of a flavone dye; **xanthorhamnin**, glucoside of rhametin; **sinigrin**, glucoside of allyl isothiocyanate; **cyanin**, glucoside of cyanidin; **delphinin**, glucoside of delphinidin; **enin**, glucoside of enidin; **pelargonin**, glucoside of pelargonidin; and **indican**, glucoside of indoxyl. It should be noted that in medical literature the term indican is used to denote indoxyl sulfuric acid but the name indican was already used by chemists to denote this glucoside of the indigo plant from which the dye indigo is made commercially, and therefore should not be used for indoxyl sulfuric acid. When injected intravenously the glucosides are excreted in the urine (Horwitt).

Horwitt: Proc. Soc. Exptl. Biol. Med. 30:949 (1933).

Perkin: Chem. Soc. Trans. 631:1180 (1893); 81:479 (1902).

Willstätter: Ann. 40:189 (1913); 48:42, 61, 83 (1915).

**Digitalis Glycosides.** **Digitalin**, **digitonin**, and **digitoxin** are derived from digitalis and are usually mixed in the tincture of

digitalis which is a heart remedy. These glucosides are very difficult to isolate. The chemistry of the aglucone is not known. **Ouabain** (*Strophanthus*) has an action similar to that of digitalis.

Sollmann: Pharmacology, fourth edition, Saunders, Philadelphia (1932).

**Sterol Glycosides.** **Phytosterolin** is a glucoside of sitosterol.

Anderson: J. Am. Chem. Soc. 46:1450 (1924).

**Saponin** is a glucoside of sapogenin and has been used in washing clothes. It is markedly cytolytic and is also used as an emetic.

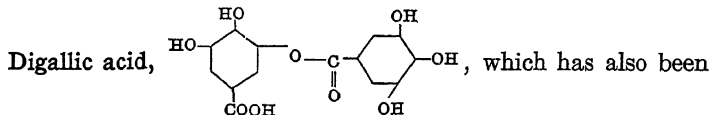
Annau and Hergloz: Arch. exptl. Path. Pharmacol. 127:93 (1927).

**Sarsa-saponin** from sarsaparilla is a glucoside of sarsapogenin and is also used as an emetic.

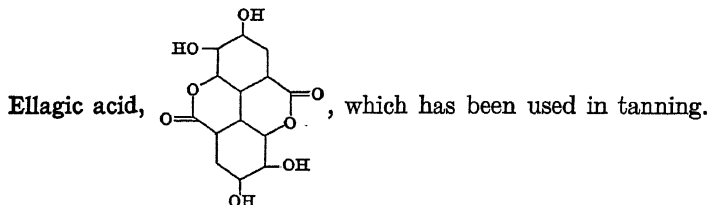
Armstrong and Armstrong: The Glycosides, Longmans, 1931.

#### TANNIN SERIES

Tannins are very complex substances and as a rule have not been obtained pure enough to crystallize. They may be derivatives of the following **depsides**:



called **tannic acid**, although the latter name has been used for more complex substances, and



**Tannins.** Two substances indicating the structure of tannins are **pentadigallylglucose** and the glucoside of **polydigalloyl-leucodigallic acid-anhydride**. These substances show the relation of the tannins to the glucosides described above, the difference being mainly in the relative amounts of glucose and the other substances, aglucones, since in the tannins there may be 5 molecules of digallic acid to 1 of glucose. Tannins combine with the proteins of hides

to form leather, and they may form much more than 50% of the total weight of the leather. Hence tannin-tanned leather is less porous than chrome-tanned leather.

Tannic acid (5%) is used in medicine to form a protective coating (by the precipitation of proteins) over denuded burns. It is also used in the determination of carbonyl-hemoglobin.

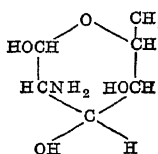
Fischer and Freudenberg: Ber. 45:915, 1116 (1912-3).

Wilson: Am. Chem. Soc. Monograph No. 12.

### AMINO SUGAR SERIES

Mixed glucosides of glucuronic acid and glucosamine are very important in physiology.

Glucosamine,

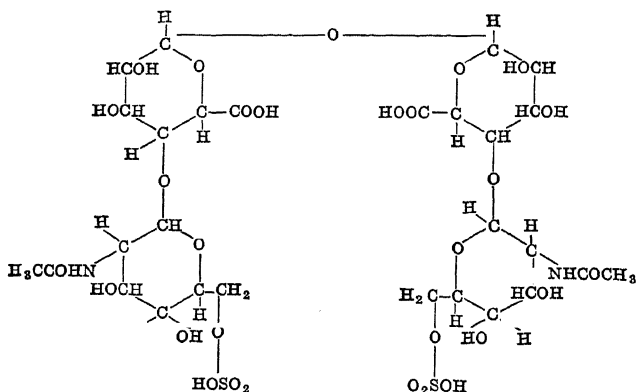


has not been found free in nature. It does not pass out in the urine, even when injected into diabetics (Baumgarten). It is formed by the decomposition of chitin

(which forms the shells of crustaceans) and of the glycoproteins.

Baumgarten: Z. exptl. Path. Ther., 2:64 (1906).

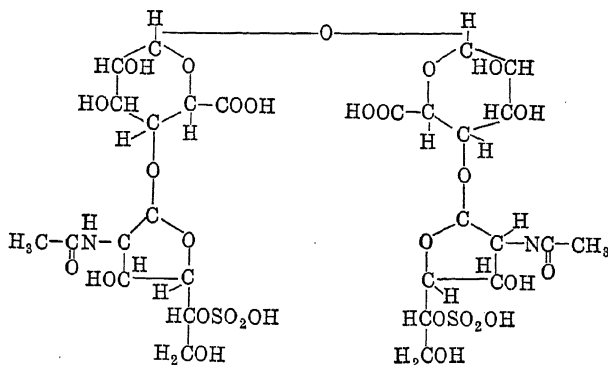
Chondroitin sulfuric acid,



is formed of sulfuric acid, glucuronic acid, and monoacetyl glucosamine. It is a constituent of cartilage.

Levene and La Farge: J. Biol. Chem. 15:69 (1913).

## Mucoitin sulfuric acid



The H<sub>2</sub>SO<sub>4</sub> is attached to the fifth carbon of the acetyl glucosamine whereas in the chondroitin sulfuric acid it is attached to the sixth carbon. The nitrogen in both cases is in peptide linkage with acetic acid.

Levene: J. Biol. Chem. 65:683 (1925).

## CEREBROSIDE SERIES

The cerebrosides have been classed as glycosides, and in the formulas which are ordinarily given for them they are glycosides or, more specifically, galactosides. They occur in brain tissue. They are related to the fats in that they contain a fatty acid, and related to the phosphatides in that they contain a nitrogenous base. It has been stated that the solubilities of the different phosphatides and cerebrosides differ, and such differences are used in their separation.

The cerebrosides occur in the myelin sheaths of the nerves. The nerve impulse is propagated by (or accompanied by) an electrolytic vortex (action current). It was shown by A. G. Mayor that the rate of the nerve impulse was proportional to the electric conductivity of the fluid bathing the nerve. The impulses of one nerve may stimulate another and similar impulses in a muscle may stimulate a nerve laid across it, as shown by the experiment in physiology of laying the nerve of one nerve-muscle preparation of the frog across the muscle of the other, stimulating the nerve of the second and causing both muscles to contract.

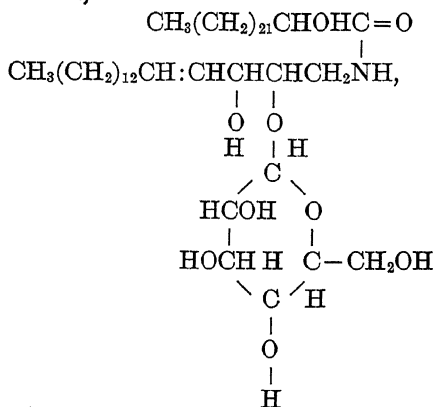
The myelin sheaths act as electric insulators but allow oxygen and carbon dioxide to pass. The nodes of Ranvier allow some electrical leakage.

The nerve may be stimulated by an induced current; thus an electric spark passing some distance from the nerve, but parallel to its length, may cause stimulation.

The fact that animals with very complex nervous systems (having many parallel nerve fibers) have greater myelinization than more primitive animals indicates that the electric insulation of the nerve fibers is important.

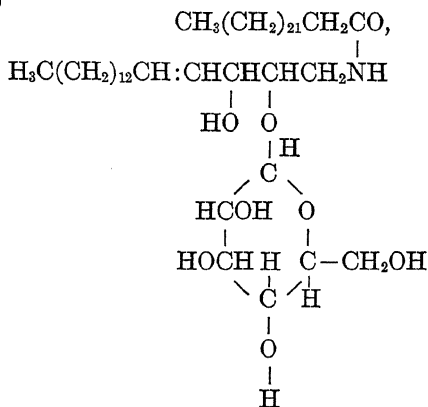
Cerebrosides form "liquid crystals," i.e., crystals without sharp angles, which may be deformed by pressure but spring back when the pressure is released. Although they do not show the sharp corners of the ordinary crystals, examination with polarized light shows that the crystal lattice is just as regular as is that of ordinary crystals. Their rounded corners are due to the fact that the influence of surface tension is greater than the force exerted by the crystal lattice. They are to be considered extremely ductile solids rather than liquids. If substances which lower the surface tension of the water in which the crystals are placed be added, local changes in surface tension cause changes in their shape, resulting in very strange shapes, which are called myelin forms.

#### Phrenosin,



contains phrenosinic acid,  $\text{C}_{24}\text{H}_{48}\text{O}_3$ , sphingosine, and galactose.

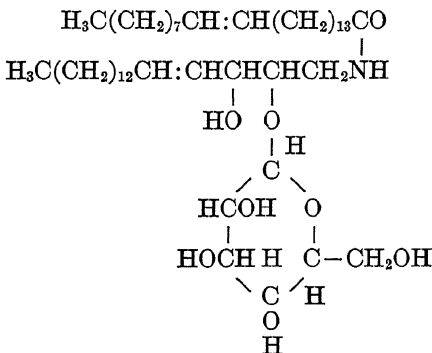
Klenk: Z. physiol. Chem. 157:291 (1926); 166:268 (1927); 174:214 (1928).

**Kerasin,**

differs from phrenosin in containing lignoceric acid,  $\text{C}_{24}\text{H}_{48}\text{O}_2$ .

Lieb and Mladenovic: Z. physiol. Chem. 140:305 (1924); 181:208 (1929).

**Nervon** differs from the above in containing nervonic acid,  $\text{C}_{24}\text{H}_{46}\text{O}_2$ .



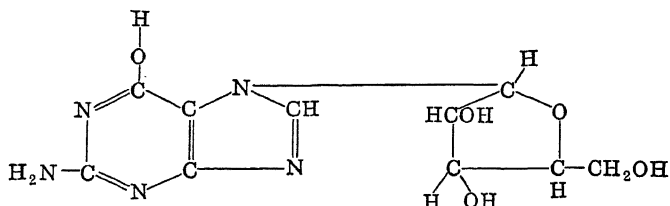
Klenk: Z. physiol. Chem. 145:244 (1925).

**NUCLEOTIDE SERIES**

A nucleotide is a compound of sugar, phosphoric acid, and a nitrogenous base. Nucleosides are nucleotides from which the phosphoric acid radicle has been removed. The sugar is either ribose or 2-deshydroxyribose.

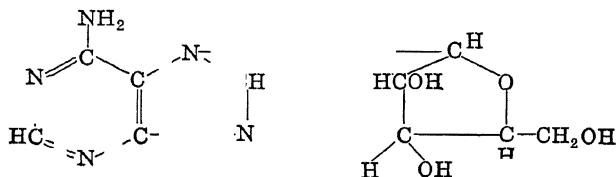
## PURINE NUCLEOSIDES

**Guanosin** is a guanine *d*-riboside.



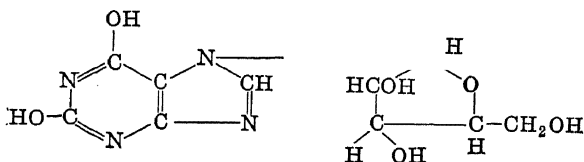
Levene and Jacobs: Biochem. Z. 28:127 (1910).

**Adenosin** is adenine riboside.



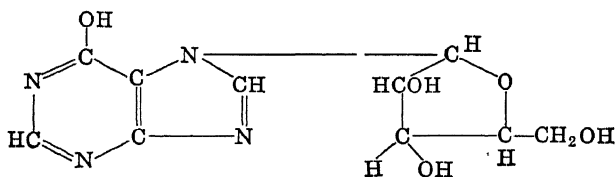
Levene and Jacobs: Ber. 42:2703 (1909); 43:3150 (1911).

**Inosin** is hypoxanthine riboside.



Levene and Jacobs: Ber. 42:435 (1909).

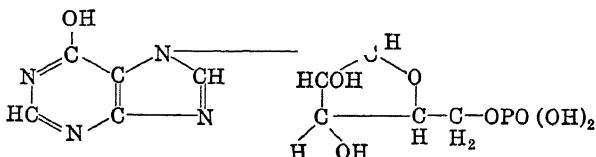
**Xanthosin** is xanthine riboside.



Jones: J. Biol. Chem. 9:169 (1911); Z. physiol. Chem. 44:1 (1911).

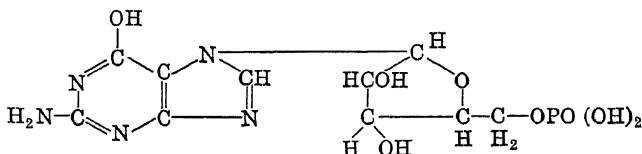
## PURINE NUCLEOTIDES

**Inosinic Acid** is inosin phosphoric acid.



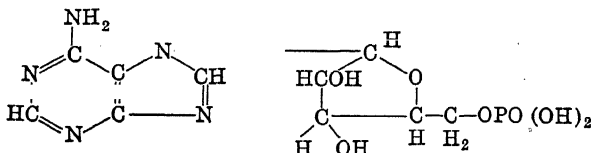
Levene: J. Biol. Chem. 81:215, 575; 83:793 (1929).

**Guanylic acid** is guanosin phosphoric acid.



Jones: Nucleic Acids, Longmans, Green & Co., New York (1920).

**Muscle adenylic acid** is adenosin phosphoric acid.

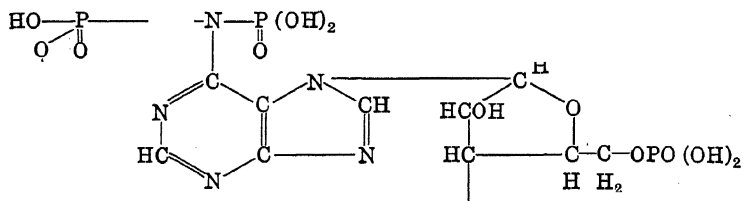


Drury and Szent-Györgyi: J. Physiol. 68:213 (1929).

**Yeast adenylic acid** has the phosphoric acid on the third carbon atom of the ribose.

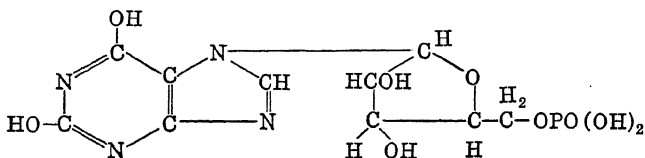
Cercedo: Am. Rev. Biochem. 2:111 (1933).

**Adenosine triphosphoric acid** occurs in muscle and on hydrolysis liberates energy.



Cercedo: Am. Rev. Biochem. 2:122 (1933).

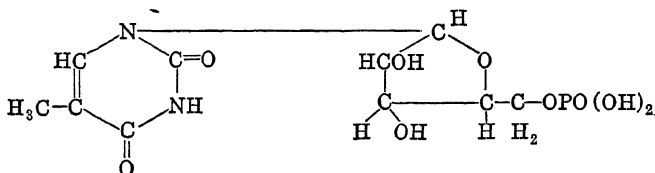
**Xanthylic acid** is xanthosin phosphoric acid formed by deamination of guanylic acid.



Knopf: Z. physiol. Chem. 92:159 (1914).

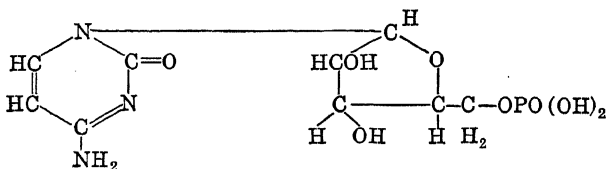
### PYRIMIDINE NUCLEOTIDES

**Thyminic acid**



Levene and Mandel: Ber. 41:1905 (1908).

**Cytidin phosphoric acid**



Thannhauser and Ottenstein: Z. physiol. Chem. 114:39 (1921).

### DINUCLEOTIDE SERIES

**Dinucleotides** have been isolated from yeast nucleic acid, one containing adenine and uracil, one guanine and cytosine, and one uracil and cytosine.

Jones: J. Biol. Chem. 20:25 (1915); 24:3 (1916); 29:111, 123 (1917); 31:39 (1917).

Levene: Ber. 42:2474 (1909); 43:3150 (1910); 44:1027 (1911); 45:619 (1912).

## NUCLEIC ACID SERIES

**Nucleic acid** is formed of two dinucleotides. Nucleic acids of the thymus, pancreas, and yeast have been more recently studied, although the nucleic acids of fish-sperm form the basis of prior work. Much discussion has arisen over the sugar element because it is the most labile group. The presence of a pentose was first proved in yeast nucleic acid but is now believed to occur in animal nucleic acids also. It has been shown that ribodesose, deshydroxyribose, occurs in thymus nucleic acid, the hydroxyl being lost from the second carbon. The bases are two purines and two pyrimidines in each molecule of nucleic acid. Some of these bases undergo changes during hydrolysis of the nucleic acid. For the digestion of nucleic acid and its split products see nucleoproteins.

Levene: Nucleic Acids, Chemical Catalog Co., New York (1931).

## DIVISION 4

## PROTIDES

**Introduction: Nitrogenous Metabolism.** The fermentation products, fats, and carbohydrates that have been considered in the above sections are compounds of carbon, oxygen, and hydrogen (although they may unite with nitrogenous bases to form phosphatides, cerebrosides, and nucleotides). The ones about to be considered contain nitrogen also. Before considering the details, it is well to outline general problems in order to have in mind what to look for under the many subheads. Nitrogenous metabolism is usually designated as protein metabolism, and the protein content of foods is often estimated as the nitrogen multiplied by 6.25 (since proteins contain an average of 16% N), sometimes without any justification.

Amino acids are classed with the proteins because proteins may be synthesized from them. When proteins are hydrolyzed by a series of proteolytic enzymes they are broken down into about 18 or 20 amino acids, and several more are found in the bodies of men and other animals. But no one has been able to substitute a mixture of known amino acids for protein in the diet of experimental animals and obtain normal growth. Rose thinks there is

an undiscovered amino acid that, if added to the mixture of known amino acids, will cause normal growth.

When a protein is hydrolyzed, there should be an increase in weight due to the water added, but this has been observed in only one or two very simple proteins. In most cases only from 50 to 90% of the weight of protein is recovered as amino acids. Therefore the biological method of analysis of proteins has been developed. This consists in feeding small mammals, usually white rats, on the purified protein as the only source of nitrogen in the diet, and observing whether normal growth takes place. Zein and gelatin are very deficient in this way and growth does not take place, but the animals lose weight. By adding different amino acids to the diet, it is found that certain ones increase the growth of the rat.

Two problems are involved in these experiments: first, what amino acids are essential to growth, and second, what amino acids are contained in the proteins fed? To find out what amino acids are essential, it would first be necessary to have a list of proteins which were deficient in the series of amino acids. Certain amino acids, such as *lysine*, one with a *benzene ring*, *tryptophan*, *histidine*, *arginine*? and *cystine*, have been shown to be deficient in certain proteins because their addition increases the rate of growth. On the other hand, with the exception of glycine, it has not been shown that the body can synthesize the remainder of the amino acids. The first achievement in this line will be a mixture of amino acids that will be a substitute for protein in the diet. It is probable that all of them except glycine are necessary and, as Rose thinks, still one more, or it is possible that chemical combinations of amino acids are necessary.

After eating a high-protein meal a large part of the products of digestion are deaminized (i.e., nitrogen removed) and their further metabolism is the same as that of the fatty acids we have already considered. This is called exogenous protein metabolism. On the other hand, the metabolism of creatine, an amino acid found in the body but not known to be a hydrolytic product of protein, is called endogenous protein metabolism (as is the total nitrogen metabolism during prolonged fasting).

The ratio of the nitrogen intake to nitrogen excretion is called the nitrogen balance, and when it is neither positive nor negative nitrogen equilibrium is said to occur.

During growth the nitrogen balance is positive, and during starvation it is negative. When the muscles grow, even in an adult, there is a positive nitrogen balance. Boothby and Duell suppose that myxedema is characterized by "deposit protein," but otherwise protein does not seem to be stored as a reserve material comparable to fat and glycogen.

Voit showed that the persons he studied (in Germany) had an average protein intake of 120 g. per day, and Atwater (in America) found that the average working man consumed 110 g. protein per day. Hindhede claimed that meat is not proper food for human beings and that the mixed vegetable diets to which milk is added is lower in protein. Since meat is expensive, this question has economic interest. Chittenden advocated a low-protein diet. Duell fed himself on a protein-free diet and reduced the urinary nitrogen to 1.75 g. per day, but some nitrogen (about 1 g.) is excreted in sweat and feces and lost from skin, hair, and nails. Since cereals are the cheapest foods high in energy content and contain about 10% protein, the lowering of protein to the least value in which nitrogen equilibrium may be obtained is not an economical but an expensive experiment.

Protein is of the same calorific value per gram as mixed carbohydrate (4.1 Cal. per g.) when burned in the body. The elimination of the nitrogen is accompanied by the loss of water from the body, but with plenty of water to drink there is no disadvantage in a high-protein diet. White rats have been fed diets in which protein was substituted for the carbohydrate and most of the fat, without detriment. The Hudson Bay Company rationed their dog teamsters 10 pounds of meat per man per day, and it has been reported that certain men (nomads) eat twice this amount.

Mitchell and Hamilton: *The Biochemistry of the Amino Acids*, Chemical Catalog Co., New York (1929).

## AMINO ACID SERIES

With few exceptions the names of amino acids end in "ine" since they are amines.

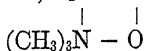
An amino acid is amphoteric, that is to say, it acts as both a base and an acid, the  $-\text{NH}_2$  group acting as a base and the  $-\text{COOH}$  group as an acid. When a hydrogen ion is added to the  $\text{NH}_2$  group, it becomes  $-\text{NH}_3^+$  (as in a dissociated base).

The acid and basic ends can be linked together to form an an-

hydride,  $\text{H}_2\text{C}-\text{CO}$ ; and when this is methylated, it becomes a

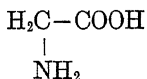


betaine. Betaine,  $\text{H}_2\text{C}-\text{CO}$ , is found in sugar beets. Many of

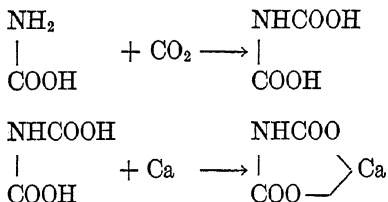


the amino acids can form methylated anhydrides (betaines).

The simplest amino acid found in protein is glycine.



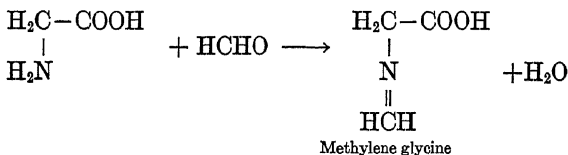
The carbamino reaction is named, however, for a simpler amino acid, carbamic acid,  $\text{H}_2\text{NCOOH}$ , which exists only in a transitory manner.



The kind of amino acid present is determined by the amount of carbon dioxide and calcium that will enter the compound. Carbamino compound formation has been suggested as a mechanism for carrying part of the carbon dioxide by the blood.

Siegfried: *Z. physiol. Chem.* 44; 46; 54.

Reaction with formaldehyde: Sørensen's titration of carboxyl groups.



Methylene glycine can be titrated, as it acts as an acid only, whereas glycine acts as either base or acid. The pH is adjusted

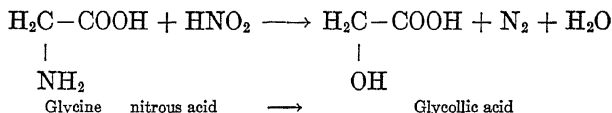
at a convenient point (the mid-point of some indicator), and formaldehyde (free from formic acid) is added. (The formic acid may be removed by adding some limestone.) It is then titrated with alkali until the *pH* returns to its original value (value before adding formaldehyde).

If  $\text{NH}_3$  is added to formaldehyde, hexamethylenetetramine is produced, so it must be removed before the Sørensen titration.

Following Fearson and Montgomery, methylene amino acids, according to the above equation, are formed in the body, followed by  $\alpha$  oxidation with the formation of  $\alpha$  ketonic acids and cyanic acid.

Sørensen: *Z. physiol. Chem.* 64:120 (1909).

Van Slyke's determination of amino nitrogen reaction with nitrous acid.



The  $\text{N}_2$  may be measured in the Van Slyke apparatus for amino nitrogen determination. In the Van Slyke determination, any  $\text{NH}_3$  present must be got rid of by aeration after making alkaline, as it reacts with  $\text{HNO}_2$ .

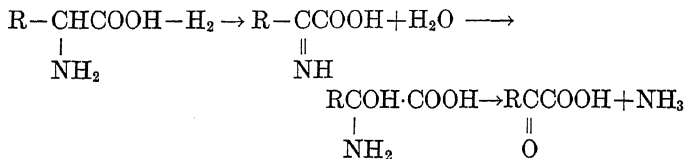
Van Slyke: *J. Biol. Chem.* 12:275 (1912).

If protein is boiled with  $\text{HCl}$  for 36 hours, almost all of it is hydrolyzed to amino acids. Always there is a little aldehyde present from sugar or other sources that condenses with tryptophan to a dark compound that is called humin.



The amino acids from proteins are  $\alpha$  amino acids, or easily derived from such. (Creatine is an  $\alpha$  guanidine acid and not an  $\alpha$  amino acid, and is found in muscle but not shown to be a constituent of proteins.)

Whereas fatty acids were shown by Knoop to undergo  $\beta$  oxidation in the body, amino acids appear to undergo  $\alpha$  oxidation, which results in simultaneous deamination and the production of  $\alpha$  ketonic acids. The production of  $\alpha$  hydroxy acids sometimes occurs but it is thought that they arise from reduction of  $\alpha$  ketonic

acids. As in the case of succinic acid, oxidation may consist in dehydrogenation followed by hydration.



**Glycine**,  $\alpha$  amino acetic acid, was first called glycoll on account of its sweet taste and the fact that it was obtained by hydrolyzing glue.

**Benzoic acid**, -COOH, is conjugated in the body with glycine to form hippuric acid, -CONHCH<sub>2</sub>COOH. It occurs in the urine of almost all animals after eating benzoic acid and was formerly used as a test for the function of the kidney. This conjugation shows that glycine may be synthesized in the body as there is not enough glycine for the amount of benzoic acid that has been taken out at one time. Some people excrete benzoic acid unless fed glycine when they excrete hippuric acid; in other words, they cannot synthesize large quantities of glycine.

Dakin has shown that  $\beta$  oxidation of  $\beta$ -hydroxy- $\alpha$ -amino acids may occur in the body; and Dakin (as well as Knoop) suggests that glycine may arise in this way.

Glycine conjugates with cholic acid to form glycocholic acid which is secreted in the bile.

The following amino acids are optically active, but only one isomere occurs in proteins. Racemic alanine is quantitatively converted into glucose in the body, but Abderhalden fed racemic amino acids to mice and found that the isomere not found in proteins is excreted.

Kingsbury and Swanson: J. Biol. Chem. 48:12 (1921).

Lewis: J. Biol. Chem. 46:73 (1921).

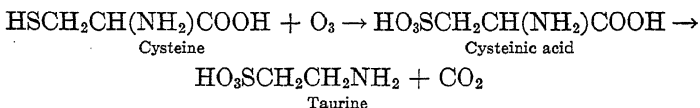
**Alanine**, H<sub>3</sub>CCH(NH<sub>2</sub>)COOH, may be formed from pyruvic acid and NH<sub>3</sub>.

Broulia: Arch. intern. Physiol. 26:169 (1926).

**Serine**, HOCH<sub>2</sub>CH(NH<sub>2</sub>)COOH, is derived from silk gum by boiling with acid, and tastes sweet. It occurs in sweat.

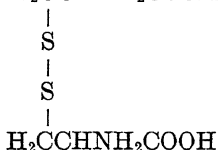
Emden and Tachau: Biochem. Z. 28:330 (1910).

**Cysteine**,  $\text{HSCH}_2\text{CH}(\text{NH}_2)\text{COOH}$ , may be oxidized with bromine and decarboxylated to form taurine.



Taurine is conjugated with cholic acid to form taurocholic acid, but the conjugation of cysteine may occur first (in the body) and oxidation and decarboxylation later.

**Cystine** (dicysteine),  $\text{H}_2\text{CCHNH}_2\text{COOH}$ , is a dehydrogenation



product of cysteine and occurs in stones in the bladder. Human hair contains 15–21%; and when cystine is deficient in the diet of rats, the growth of the hair is retarded (Lewis). Cystine is converted into glucose in the diabetic.

Cystine or cysteine is necessary in nutrition, and related nitrogen-free-S-S-compounds while oxidized in the body (Rose) cannot be substituted so as to maintain normal growth of rats (Lewis). Abderhalden traced cystinuria through 3 generations. Robson traced cystinuria through 2 generations with 6 cases in the first generation and 5 in the second and concluded that it is hereditary. He fed a cystinuric rat 20 g. of cystine in 4 days without increasing the cystine in the urine although there was enough extra sulfate in the urine to indicate that 85% of the cystine was oxidized in the body. It is stated that the cystine may be combined with some unknown substance in the urine. Death from an overdose of cystine may result from precipitation of cystine not only in the kidneys and bladder but also in the spleen, mesenteric lymph nodes, liver, and intestinal walls. Cystine is nephropathic because of its 2 carboxyls.

Abderhalden: *Z. physiol. Chem.* 38:557 (1903).

Robson: *Biochem. J.* 23:138 (1929).

**Methionine**,  $\text{H}_3\text{CSCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ , occurs in proteins. It is found in the blood.

Barger and Coyne: *Biochem. J.* 22:1417 (1928).

**Valine**,  $(\text{CH}_3)_2\text{CHCH}(\text{NH}_2)\text{COOH}$ , occurs to the extent of 7.9% in casein and derives its name from plants of the genus *Valeriana*. It does not yield sugar or ketone bodies in the diabetic.

Vickery: J. Biol. Chem. 65:657 (1925).

**Norleucine**,  $\text{CH}_3(\text{CH}_2)_3\text{CH}(\text{NH}_2)\text{COOH}$ , discovered by Abderhalden, has been isolated from proteins by Schmidt.

Czarnetzky and Schmidt: J. Biol. Chem. 97:333 (1932).

**Leucine**,  $(\text{CH}_3)_2\text{CHCH}_2\text{CH}(\text{NH}_2)\text{COOH}$ , occurs to the extent of 27% in zein, and as crystals in carcinoma and tubercular lymph nodes, and is often found in the urine.

The normal metabolism of leucine is said to include oxidative deamination and decarboxylation to isovaleric acid and then to be demethylated and oxidized to  $\beta$ -hydroxybutyric acid. In alcoholic fermentation it yields isoamyl alcohol. It is said to change negative serum to positive in the Wassermann reaction.

Leiter: Z. Immunitäts. 30:105 (1920).

**Isoleucine**,  $\text{H}_3\text{CCH}_2\text{CH}(\text{CH}_3)\text{CH}(\text{NH}_2)\text{COOH}$ , does not yield glucose in the diabetic.

Robinson: J. Am. Chem. Soc. 33:564 (1911).

#### DICARBOXYLIC MONO AMINO ACIDS

Dicarboxylic mono amino acids damage the kidney if given in excessive amount. They occur in large amounts in some of the proteins.

**Aspartic acid**,  $\text{HOOCCH}_2\text{CH}(\text{NH}_2)\text{COOH}$ , is found in proteins and is named from asparagus. When injected intravenously in dogs, 2 g. per kg. is mildly nephropathic. Three of the carbon atoms are transformed into glucose in the diabetic (Lusk).

Keenan: J. Biol. Chem. 62:163 (1924).

**Glutamic acid**,  $\text{HOOCCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ , as the monosodium salt, is the basis of Japanese and Chinese sauces that allow Buddhists to enjoy the flavor of meat without eating the forbidden flesh. It is less nephrotoxic than glutaric acid. In the diabetic 3 carbon atoms are converted into glucose (Lusk).

Abderhalden claims that if aspartic and glutamic acids are added to a mixture of amino acids without them, better nutrition results.

Ikeda electrodialed a neutral, wheat-gluten hydrolysate with zinc anode, collecting the zinc salts of glutamic and aspartic acids in the anode compartment.

Foster and Schmidt: J. Biol. Chem. 56:545 (1923).

Ikeda and Suzuki: U. S. Patent No. 1,015,891, Jan. 30, 1912.

**$\beta$ -Hydroxyglutamic acid**,  $\text{HOOCCH}_2\text{CH}(\text{OH})\text{CH}(\text{NH}_2)\text{COOH}$ , was discovered by Dakin, and Calvery found 1.36% in egg albumin, but some other workers have failed to obtain the natural product. It may be prepared synthetically from glutamic acid.

Calvery: J. Biol. Chem. 94:630 (1932).

Dakin: J. Biol. Chem. 13:398 (1919).

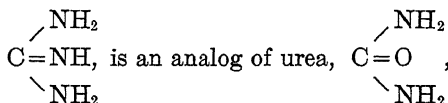
### BASIC AMINO ACIDS

**Lysine**,  $\text{H}_2\text{N}(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{COOH}$ , one of Kossel's hexone bases, will increase the growth of a young rat when added to a diet deficient in lysine.

Lysine is not transformed into glucose or ketone bodies in diabetic animals (Dakin).

Bunny and Rose: J. Biol. Chem. 76:52 (1928).

**Arginine**,  $\text{H}_2\text{NC}:(\text{NH})\text{NH}(\text{CH}_2)_3\text{CH}(\text{NH}_2)\text{COOH}$ , one of Kossel's hexone bases, has a guanidine nucleus. Guanidine,



and was obtained from the fertilizer, guano. Arginine has been found to be essential in nutrition (denied by Scull and Rose).

Three of the carbon atoms of arginine are transformed into glucose in the diabetic (Dakin).

Arginine is split by arginase into urea and **ornithine**,  $\text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ , an amino acid which does not occur in proteins but, as its name implies, is found in birds. Since the liver contains arginase, ornithine may be an intermediate in the metabolism of arginine.

Hyde and Rose: J. Biol. Chem. 84:535 (1929).

**Creatine** (name means flesh),  $\text{HN}=\text{C}-\text{NH}_2$  , has  
 $\quad \quad \quad |$   
 $\quad \quad \quad \text{N}(\text{CH}_3)\text{CH}_2\text{COOH}$   
 never been produced by boiling protein with acid. It is found in

every muscle of the body, striated muscle containing about 0.4%, rabbit muscle 0.5%.

It may be derived from arginine by oxidative deaminization and two successive decarboxylations, but the feeding of arginine to a man up to the equivalent of 1 g. creatine daily for 2 months has not been shown to increase the production of creatine (Rose), and no increase occurs in the dog (Lewis) whereas there is an increase in the pig (Steenbock).

Normal blood contains 3-7 mg. creatine per 100 cc., but it is found in the urine only in children, women during menstruation and after parturition, eunuchs, fasting persons, and persons with muscular dystrophy, myasthenia gravis, exophthalmic goiter, tumor cachexia, and diabetes, but some of these are doubtful. In determining creatine, creatinine is determined on an aliquot sample and another aliquot is acidified and autoclaved to change creatine to creatinine. Ketone bodies cause low readings of creatinine; and as these are removed by autoclaving, the creatinine values are increased; and the increase is reported as creatine.

There may be a relation between creatinuria and low blood pressure or lack of oxygen in the muscles.

When a moderate amount of creatine is fed to a normal man, the muscles absorb it from the blood to such an extent that none passes into the urine. Chanutin and Silvette showed that it was stored in the muscles and liver, but some was destroyed. Benedict and Osterberg fed a dog about 0.5 g. creatine per day for 70 days. An increased excretion of creatinine started about a week after the feeding began. Nearly 40% of the creatine was excreted, and about 18% was changed to creatinine and excreted leaving 42% unaccounted for (retained or destroyed).

Benedict and Osterberg: *J. Biol. Chem.* 56:229 (1923).

**Phosphocreatine**,  $\text{HN}=\text{C} \begin{cases} \text{NHPO}(\text{OH})_2 \\ \text{N}(\text{CH}_3)\text{CH}_2\text{COOH} \end{cases}$ , occurs in the muscles and accounts for some of the phosphoric acid and most of the creatine in the muscles. Glucose phosphate is present in muscle; and when the glucose is changed to lactic acid, phosphoric acid is liberated. The creatine combines with some of the phosphoric acid. It is represented by phosphoarginine in lower animals.

Since creatine is not a constituent of proteins, the occurrence

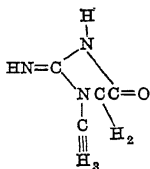
of creatine in muscle tissue has been something of a mystery. The discovery of about 0.5% phosphocreatine in muscle by Fisk and Subbarow, which had previously been considered creatine and phosphoric acid, throws new light on the mystery.

During muscular excitation or contraction, phosphocreatine undergoes hydrolysis, whereas synthesis takes place during the recovery period. The union of phosphorus with nitrogen is unstable and makes this substance adapted to change with physiological conditions. White muscle contains more than red muscle.

Meyerhof supposes that the energy for the synthesis of phosphocreatine is derived from the hydrolysis of adenosine triphosphoric acid.

Fiske and Subbarow: *J. Biol. Chem.* 81:629 (1929).

### Creatinine,



the internal anhydride of creatine was discovered by Liebig in normal urine in 1847 and first synthesized by Volhard in 1868. In the urine, the amount depends upon the muscular development of the individual.

Men excrete an average of 25 mg. (15–33 mg.) per kg., women average 18 mg. (10–27 mg.) per kg., in 24 hours, and children average small amounts (this is known as the creatinine coefficient and is very constant from day to day and is used practically to mark off daily periods in urine collection).

It occurs also in blood, about 1 mg. of creatinine in 100 cc. blood in a normal person. When creatinine rises above 3.5 mg. per 100 cc. blood, it indicates nephritis. During nephritis, if it reaches 5 mg., the case is very serious; but it may go to 25 mg. before death. Creatinine is the last of the metabolites to increase and is considered much more serious than increased urea or uric acid. When the amount of creatinine is greater than uric acid, it is very serious.

There is more creatine than creatinine in the blood. The creatinine content of the blood is apparently independent of the diet. The corpuscles are richer in creatinine than the plasma. It also has been found in the amniotic fluid.

It is derived from creatine probably in the kidney. The excitation of the nervous system increases the creatinine excretion. The creatinine coefficient increases during mortal agony.

Feeding or subcutaneous injection of very large amounts of creatine leads to an increase in creatinine. The creatinine coefficient drops below normal in pulmonary tuberculosis. Alcohol decreases the amount of creatinine excreted.

The creatinine seems to act independently of creatine during creatinuria. For example, creatinine remains constant or decreases during starvation accompanied sometimes by the presence of creatine; it increases with no increase in creatine in fever; it decreases with increased creatine in exophthalmic goiter or in diabetes; and it has been found that in a thyroidectomized dog there is an increased creatine excretion with no change in creatinine. Creatinine is non-toxic; and when administered orally or subcutaneously, it is excreted as such.

Benedict and Allen: *J. Biol. Chem.* 46:21 (1921).

Beumer and Iseke: *Berlin klin. Wochschr.* 57:178 (1920).

Burger: *Z. Exptl. Med.* 9:262 (1919).

Mellanby: *J. Physiol.* 26:477 (1907).

Meyers and Killian: *Am. J. Med. Sci.* 157:674 (1919).

McLaughlin and Blunt: *J. Biol. Chem.* 58:285 (1923).

Neubauer: *Münch. med. Wochschr.* 61:857.

Rabinowitch: *Can. Med. Assoc. J.*, 11:320 (1921).


Rose, Ellis, and Helming: *J. Biol. Chem.* 77:171 (1928).


Shiver: *Chem. Rev.* 6:419 (1929).

Taylor: *Biochem. J.* 5:362.

Weinberg: *Biochem. J.* 15:206 (1921).

#### AROMATIC AMINO ACIDS

**Phenylalanine**,   $\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ , is completely burned in the body of a normal person although phenylpropionic acid is merely oxidized to benzoic. Phenylalanine is essential in the diet unless it contains:

**Tyrosine**,  $\text{HO}$ -  $\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ , one of the earlier amino acids to be discovered, being obtained by fusion of cheese with alkali, dissolving in water and allowing the dissolved mixture to stand, from which tyrosine crystallizes out since it is very slightly soluble in cold water. Several color tests have been devised for showing the presence of tyrosine in protein.

Xanthoproteic test is executed by simply adding nitric acid to the protein or protein solution and then making it alkaline. The

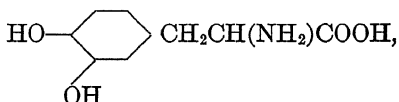
tyrosine is changed to a nitrated phenyl ring which acts as an indicator and shows a stronger yellow color in alkaline solution.

Phenylalanine is not nitrated in the cold or on short boiling; in fact, an autoclave is desirable for its nitration. Therefore, it does not react in a practical manner to this test.

Millon's test depends on nitrating tyrosine and then adding mercury which gives a pink color rather than a yellow one. Millon's reagent is made by simply adding nitric acid to mercury, waiting till it stops fuming, and then adding water. Phenol will give this test.

Since most substances containing the benzene ring pass through the body without the ring being broken, the complete oxidation of phenylalanine and tyrosine in the body has given rise to much speculation as to the manner in which the ring is broken. Alpha oxidation of these  $\alpha$  amino acids would give rise to phenylacetic or *p*-hydroxyphenylacetic acid, neither of which is oxidized in the body. Hence some other point must be attacked.

3-4-Dihydroxyphenylalanine, dopa,



is produced by the action of tyrosinase on tyrosine.

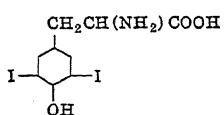
Dopa-oxidase oxidizes dopa to the corresponding quinone which undergoes intramolecular change to 5-6-dihydroxydihydroindole-2-carboxylic acid which is oxidized to the corresponding quinone (Raper). This last substance is red but is transformed to a colorless substance which changes to a black (melanin).

Since melanin is of higher molecular weight than the amino acids from which it arises, the formation of melanin does not solve the problem of the combustion of phenylalanine and tyrosine in the body. Dakin injected phenylalanine into a rabbit and recovered it in the urine, but that might occur with any amino acid.

It is thought that *p*-hydroxyphenylpyruvic acid is an intermediate in the combustion of both phenylalanine and tyrosine, but the mode of the breaking of the ring is unknown.

Block and Schaaf: *Biochem. Z.* 162:181 (1925).

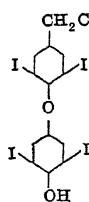
Raper: *Biochem. J.* 21:89 (1927).

**Diiodotyrosine,**

is found in thyroglobulin from the thyroid gland, spongin from sponges, gorgonin from gorgonians, and

from other marine organisms. Its special significance is not known, but it is thought to be a building stone of:

Foster: J. Biol. Chem. 83:345 (1929).

**Thyroxine,**

the hormone of the thyroid gland which is produced by the alkaline hydrolysis of thyroglobulin. It has not been obtained from any other source except the thyroglobulin in the thyroid gland. Even when large doses are in-

jected intravenously, no trace can be recovered.

If 1 mg. of thyroxine is injected into an adult, in 50 hours the basal metabolic rate is increased about 3%. The effect gradually

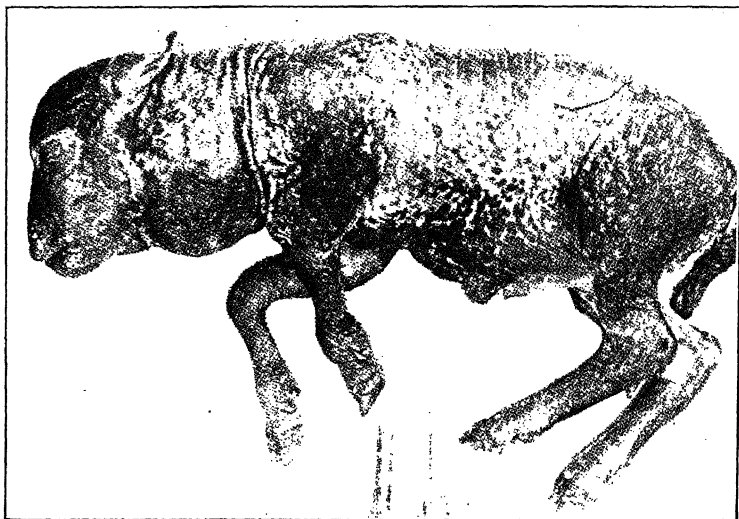


FIG. 49. Goitrous lamb. Montana Agricultural Station Bulletin.

dies off during the succeeding weeks. If excised tissue is put into a solution of thyroxine and allowed to remain even for 50 hours, its metabolic rate is not increased; but if thyroxine is injected

into an animal and the tissue cut out 50 hours later, its metabolic rate is increased. What change has taken place in the thyroxine injected into the blood-stream is a problem for future research. At present the study is not chemical but biological. Kendall, its discoverer, showed that its potency is easily destroyed by reduction, which removes the iodine.

Gudernatsch observed that thyroid increased the differentiation of tadpoles and made them metamorphose into frogs at more than 10 times the normal rate, and this has been shown to be due to thyroxine. By the tadpole test potent substances have been obtained from other parts of the body besides the thyroid.

Lack of thyroxine causes increased growth of the thyroid and produces a condition which, if arising in the fetus and continuing, leads to cretinism, or if arising in adult life, to some of the symptoms of cretinism including myxedema. The cretin's mentality does not develop, and his body remains more or less retarded in anatomical development. Owing to the fact that there are different degrees of deficiencies besides total absence of thyroxine, all gradations of cretins are known. In domestic animals cretins are often born dead or die at an early age (fig. 49). Stockmen of Montana and eastern Washington have to supply iodine to prevent loss of stock by cretinism, since thyroxine is 65% iodine.

The same has been said of Michigan, Wisconsin, and other states. In experimental animals, lack of iodine causes increased growth of the thyroid (fig. 50).

An amphibian without thyroxine remains in the larval form during its whole life. The axolotl was thought to be a new species

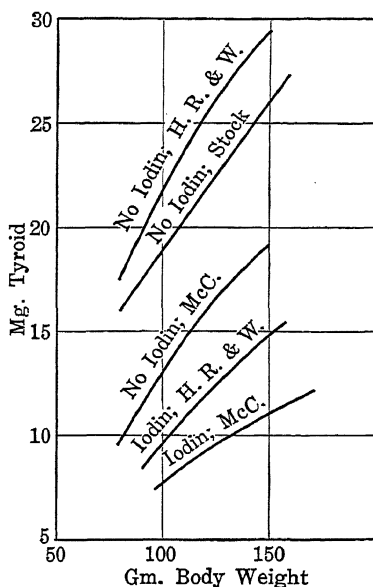


FIG. 50. Growth of thyroid with iodine. Physiological Reviews.

because it reproduced in the larval stage and was never seen in the adult stage in its native home in Mexican lakes. When taken to Paris where there was more iodine in the water, the axolotl metamorphosed into the tiger salamander. This was one of the most sensational occurrences that ever happened in biology, but its chemistry was not at all understood until years later.

The pituitary gland is very high in iodine, and its chemistry has not been worked out, but it stimulates the thyroid.

The administration of an overdose of thyroxine produces an increase in pulse-rate. If the heart is cut out 50 hours after the injection of thyroxine, it continues to beat at the increased rate; but if the heart of a normal animal is cut out and then treated with thyroxine, its rate is not increased.

The attempt to produce exophthalmos (which was supposed to be due to too much thyroxine) by the administration of thyroxine has failed. However, M. Kunde found that rabbits whose thyroid glands were cut out and were thus made myxedematous for many months sometimes developed a slight exophthalmos. When they were then given thyroxine, the exophthalmos increased for a short time and then disappeared. Exophthalmos has been produced in thyroidectomized guinea-pigs by anterior pituitary (Marine and Rosen). No one has shown that exophthalmic goiter has anything to do with an over-production of thyroxine. The only similarity in the two conditions is the increase in basal metabolic rate. If normal or diseased thyroid is cut out leaving a small piece, it will regenerate. The chemical constitution of thyroxine was worked out by Harington.

Cameron and Carmichael: *J. Biol. Chem.* 46:35 (1921).

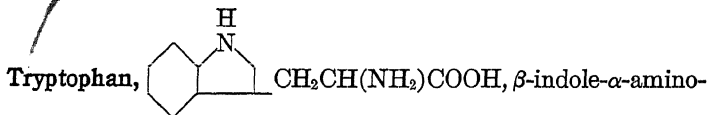
Harington: *Biochem. J.* 20:293, 300 (1926).

Kendall: *J. Biol. Chem.* 29: Proc. xxix (1917); 80:357 (1928).

Marine: *Physiol. Rev.* 2:521 (1922).

Marine and Rosen: *Proc. Soc. Exptl. Biol. Med.* 30:901 (1933):

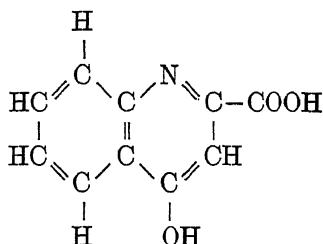
#### HETEROCYCLIC AMINO ACIDS



propionic acid, was discovered by following a color reaction on proteins. Adamkiewitz showed that glacial acetic acid gave

a color reaction with protein and  $\text{H}_2\text{SO}_4$ . Hopkins and Cole showed that glyoxylic acid (in the acetic) was the basis of the reaction. Neumeister had already applied the name tryptophan to a hypothetical color-producing substance associated with the decomposition of proteins during pancreatic digestion. Hopkins and Ackroyd attempted to raise mice on amino acids instead of proteins. In attempting to isolate tryptophan, they showed that it is destroyed by hydrolyzing protein with acid and succeeded in isolating it from a tryptic digest. Zein, the chief protein of corn meal, contains no tryptophan, and growth on it ceases unless tryptophan is added. Rose has shown that  $\alpha$ -keto- $\beta$ -indole propionic acid may be substituted for tryptophan in the diet. Therefore, the animal can synthesize tryptophan from it by reduction and the addition of ammonia. Tryptophan gives the xanthoproteic but not Millon's test.

#### Kynurenic acid

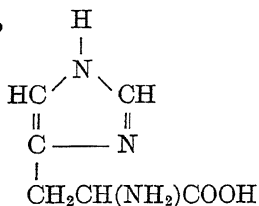


is an intermediate in the metabolism of tryptophan in dogs and rabbits. When fed to man, kynurenic acid is utilized; but according to Asayama, it cannot replace tryptophan in the diet. Kynurenic acid occurs in dog's urine, but that might be considered a failure of tryptophan metabolism, as acetoacetic acid is considered a failure of fat metabolism. It has been stated, in connection with the metabolism of tryptophan, that the indole ring is disrupted, leaving a benzene ring with nitrogen in side chain and the quinoline ring is completed from the side chain.

Hopkins and Cole: *J. Physiol.* 27:418 (1901); 29:451 (1913).

Mendel: *J. Am. Med. Assoc.* 64:1539 (1915).

Robson: *Biochem. J.* 22:1157 (1928).

**Histidine,**

$\alpha$ -amino- $\beta$ -imidazole propionic acid, one of Kossel's hex-one bases, is widely distributed in proteins especially in some histones, constituting 11% of globin. Ekroyd and Hopkins thought that histi-

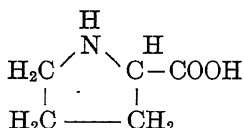
dine and arginine were interconvertible, only one being necessary in nutrition. Rose could not confirm this. Rose found, however, that  $\alpha$ -keto- $\beta$ -imidazole propionic acid could be substituted as well as racemic imidazole lactic acid. Edelbacher claims that histidine may be changed over to glutamic acid in the body. Dakin found histidine ketogenic.

Urocanic acid (see below) occurs in dog's urine and is said to be derived from histidine.

Rose observed that removal of histidine from the diet reduced uric acid output 50%. Feeding histidine leads to increase in uric acid in human urine, and to allantoin (see below) in the urine of rats and other animals.

Trimethylthiol-histidine (see below) has not been shown to be a constituent of proteins although it is increased by certain diets.

Rose and Cox: J. Biol. Chem. 61:747 (1924); 64:325 (1925); 68:217, 769, 781 (1926).

**Proline,**

pyrrolidine 2-carboxylic acid, is not an  $\alpha$  amino acid; but it is supposed that it can be formed from hydroxyglutamic acid by closing the ring, since Abder-

halden synthesized pyrrolidine carboxylic acid from glutamic acid. Proline does not react with nitrous acid or ninhydrine. In a diabetic 3 of its carbon atoms are converted into glucose, showing that the ring may be disrupted.

Dakin: J. Biol. Chem. 13:513 (1913).

**Hydroxyproline** is an amino acid of doubtful constitution. The hydroxyl group is in either the  $\alpha$  or  $\beta$  position. It is widely distributed, especially in scleroproteins. It is converted into glucose in the diabetic (Dakin).

These pyrrolidine compounds by dehydrogenation may be changed to pyrrol compounds and so be building stones of hematin.

If 3 drops of 2% octanol is placed in a test tube with a small amount of protein and a few drops of water added followed by 0.5 g. carbonate-free  $\text{Na}_2\text{O}_2$ , and the contents mixed and heated until nearly dry, cooled, and 2 cc. 5N HCl added and heated on the water bath, an amber rose color denotes about 1 mg. of hydroxyproline (Morse).

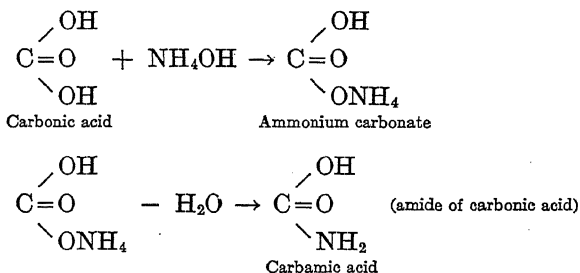
Frankel and Jellinik: *Biochem. J.* 13:592 (1922).

Morse: *J. Biol. Chem.* 100:373 (1933).

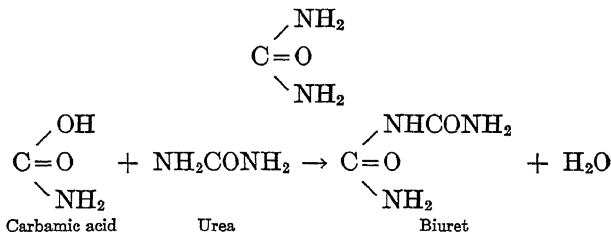
## PEPTIDE SERIES

Most amino acids are linked in the way proposed by Kossel and worked out by Emil Fischer, known as the imide linkage (also called peptide linkage).

This linkage occurs in biuret.



This is the simplest amino acid. The amide of carbamic acid is urea,



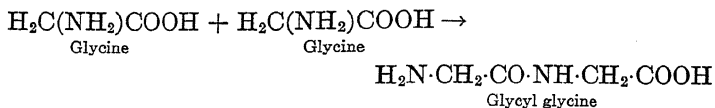
When a very weak solution of copper sulfate is added to biuret, a very strong dye is formed. Copper salts alone are colored, but an invisible trace of them is all that is necessary to obtain this reaction. However, the solution must first be made alkaline. This is a convenient test for the  $-\text{CONH}-$  group in proteins

or peptides containing three or more amino acids. Carbamic acid does not occur in them, however.

Rising and Johnson: *J. Biol. Chem.* 80:709 (1928).

#### DIPEPTIDE SERIES

**Glycyl glycine**,  $\text{H}_2\text{N}\cdot\text{CH}_2\cdot\text{CO}\cdot\text{NH}\cdot\text{CH}_2\cdot\text{COOH}$ , is formed by the union of two glycine molecules:



Any two amino acids may be linked to form a dipeptide. Dipeptides are not hydrolyzed by pepsin or trypsin but only by dipeptidases. Prolylglycine is hydrolyzed by prolinase (Grassman).

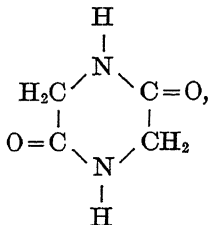
Peptides of the naturally occurring amino acids are hydrolyzed by peptidases; those containing one or more optical isomeres of the natural amino acids usually resist hydrolysis.

When proteins are acted on by the enzyme pepsin in the right amount of acid, they are broken down to peptones (polypeptides).

Fischer has joined as many as 18 amino acids to form polypeptides by the imide linkage, and Abderhalden 19, having 3875 possible isomeres.

Abderhalden: *Neuere Ergebnisse der Eiweisschemie*, Jena (1909).

The first step in many of these syntheses was the formation of: **2,5-Diketopiperazine** (2,5-piperazinedione),



is a reduced heterocyclic compound. Any two  $\alpha$  amino acids may be united to form this ring, the portion not represented in glycine forming a side chain attached to the  $\text{CH}_2$  group. There is a question whether or not proteins contain this ring. Other groups

may by peptide linkage be attached to a diketopiperazine if it is made of dicarboxylic or diamino acids, and in this way proteins of high molecular weight might be built. Abderhalden has made an extensive study of these rings. Waldschmidt-Leitz claims that these cyclic compounds are not found in proteins, as most proteins may be split by digestive enzymes whereas these rings cannot be split by digestive enzymes. Levene claims that diketopiperazine rings occur in the scleroproteins: keratin and fibroin, which are not split by digestive enzymes.

When proteins are hydrolyzed by acid, synthesis is going on at the same time, humin being formed. This synthesis is said to be due to aldehydes present. The synthesis of these cyclic compounds (diketopiperazines) is said to occur also, and their presence in the protein hydrolysate is no proof of their occurrence in proteins.

Abderhalden: *Z. physiol. Chem.* 178:156 (1928); 151:114, 148 (1926); 140:92 (1924).

Waldschmidt-Leitz: *Collegium* 543 (1928).

**Glycylthyroxine**, m.p. 188–190 d., is very poorly soluble in water and has similar physiological properties to thyroxine.

**dl-Alanylthyroxine**, m.p. 195–200 d., is very poorly soluble in water and has physiological properties similar to those of thyroxine.

Harington: *The Thyroid Gland*, London (1933).

**Carnosine**,  $\beta$ -alanyl-histidine, has been found in muscle. It is a dipeptide of histidine and  $\beta$ -alanine, an amino acid not found in proteins.

Clifford: *Biochem. J.* 15:400, 725 (1921); 16:341, 792 (1922).

When protein is digested, in one stage it is broken down to a mixture of polypeptides, known as peptone. When food is chewed in the mouth, it is mixed with a neutral solution of saliva, and then passes into the stomach. The stomach secretes 0.1 *N* HCl solution. Dog's gastric juice contains a higher percentage of HCl (almost 50% higher) than humans'. Few people are unable to secrete gastric juice. This condition usually occurs in people with pernicious anemia and is known as achylia, and has the same effect on one as absence of the stomach. Pavlov once organized a "dairy" supplying gastric juice. The gastric juice was led

out from a Pavlov pouch of a dog's stomach. A number of such dogs supplied gastric juice to patients in need of it. Gastrectomy produces anemia. According to a group of investigators, a dipeptide, hydroxyproline-hydroxyglutamic acid, in liver extract was reported to cure pernicious anemia. Felix says the active fraction of liver does not contain peptide. A gastric digest of meat will cure pernicious anemia. It is an attractive suggestion that pepsin splits protein into peculiar peptides, one or more of which are necessary in nutrition, which are not the same as the peptides produced by tryptic digestion.

When acted on by polypeptidases of erepsin, the polypeptides may be broken down to dipeptides, and these acted on by dipeptidases of erepsin are broken down to amino acids. Before the action of other enzymes takes place, some of the polypeptides may be absorbed. The dipeptides especially may escape the action of enzymes, but when insulin (a polypeptide) is eaten most of it is destroyed before being absorbed. Hydroxyproline-hydroxyglutamic acid is obtained from the liver, which contains proteolytic enzymes. The function of the stomach is to break down proteins to polypeptides (peptones), and apparently one cannot get along without it unless enough of these are taken in with food.

After gastrectomy a patient usually dies within 6 years, and in those cases in which a blood-count was made anemia was found. Ivy has kept dogs alive for considerable periods after gastrectomy, but it has not been shown that polypeptides were absent from the diet. Since enzymes are secreted into the gut capable of hydrolyzing the polypeptides down to amino acids, many investigators believe only amino acids are absorbed. If this were the case, ricin, a toxalbumin, should not be toxic since it does not contain amino acids other than those found in ordinary proteins. Polypeptides exist in the gut as well as in the blood, and the simplest hypothesis is that they pass from the gut into the blood. The fact that under ordinary conditions only a small amount of insulin passes may be explained by its high molecular weight. It seems probable that peptides of lower molecular weight are absorbed more rapidly.

Felix and Frühwein: *Z. physiol Chem.* 216:173 (1933).

Finney and Reinhoff: *Arch. Surgery* 18:140 (1929).

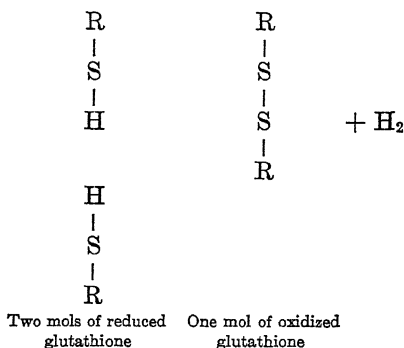
Minot and Murphy: *J. Am. Med. Assoc.* 87:470 (1926); 89:759 (1927).

## TRIPEPTIDE SERIES

Pepsin does not hydrolyze tripeptides. Trypsin-kinase hydrolyzes tripeptides containing tyrosine but not polypeptides of glycine and leucine only. These latter are hydrolyzed by erepsin.

**Glutathione** is a tripeptide of cysteine, glutamic acid, and glycine. It occurs in every tissue of the body and in blood, and some is metabolized by the mammary gland in milk secretion. It has to do with oxidation and reduction.

Wieland's theory of oxidation: Oxidation of a substance in the body is not the combining of oxygen with the substance but the taking away of hydrogen. The hydrogen is united with oxygen to form water by the respiratory enzyme, which contains iron.



The hydrogen may be furnished by succinic acid and other substances considered above. Glutathione takes away two hydrogen atoms and thus oxidizes foodstuffs. Glutathione has been especially studied by Hopkins.

Hopkins: Biochem. J. 15:286 (1921); 19:787 (1925).

## POLYPEPTIDE SERIES

Blood serum contains 0.05% polypeptide nitrogen (Herzfeld).

**Insulin**,  $(\text{C}_{45}\text{H}_{69}\text{O}_{14}\text{N}_{11}\text{S})_2$ , is an important polypeptide. It is not known how many amino acids occur in it; those reported are arginine, histidine, lysine, cystine, tyrosine, and leucine. Diabetes might be considered as due to absence of the right enzymes to break down protein into insulin or to build it up from amino acids. The pancreas contains trypsinogen, which, when acted on by

enterokinase, forms trypsin (or in the language of Waldschmidt-Leitz trypsin unites with enterokinase to form trypsin-kinase), which acts on insulin and destroys its activity. Insulin can be extracted from the pancreas after stopping the enzyme action by placing the pancreas in alcohol or acidulated water at a low temperature. It is difficult to separate it from other proteins. Abel produced optically active, crystalline insulin with isoelectric point pH 5.55-5.65.

Abel and collaborators: J. Pharmacol. 31:65 (1927); 32:367, 387 (1927-8); 33:497 (1928); 36:115 (1929).

**Tri or Tetrapeptide**, containing thyroxine, is soluble in water.  
**Heptapeptide**, containing thyroxine, is soluble in water.

**Polypeptide of thyroxine and 19 other amino acids** (soluble in water) has been prepared and has the same physiological effect as thyroxine intravenously. The greater solubility may be the cause of the greater effect of the polypeptide over thyroxine by mouth.

Harington: The Thyroid Gland, London (1933).

**Secretin**, the hormone discovered by Bayliss and Starling which causes the pancreas to secrete, is said to be a polypeptide.

Mellanby: J. Physiol. 66:1 (1928).

Still: Am. J. Physiol. 91:405 (1929-30).

Polypeptidases are divided into (1) amino-polypeptidases with a special prolinase and (2) carboxy-polypeptidases with special tyrosine and leucine polypeptidases. Aside from the polypeptides, the chemistry of proteins is not known. They have been classed according to (1) solubility, (2) origin, and (3) hydrolytic products. The species of organism is first ascertained and the protein named for its origin, solubility, and hydrolytic products.

Polypeptides are not coagulable by heat or precipitated by saturating their solutions with ammonium sulfate. In this their definition exactly coincides with that of peptones. Many polypeptides have been isolated from **peptones**, and the fact that many mixtures called peptones contain proteose does not warrant a distinction between peptones and polypeptides. Peptones produce shock when injected. Witte peptone is made from fibrin.

Waldschmidt-Leitz and collaborators: Ber. (B) 60 (1927); 299 (1928).

### PROTEINS OF HIGH MOLECULAR WEIGHT

It has been stated that proteins are very unstable but since wool and silk are proteins and shoes are made of protein (rawhide is protein and leather is a tannin-protein compound), one can readily see that they are quite stable in the absence of hydrolytic enzymes. Eggs, for instance, spoil because of enzyme action on protein, and they contain such enzymes and others are secreted by bacteria.

Proteins are very high in molecular weight. Casein, the protein of milk, and edestin, the protein of cottonseed, are sold commercially.

If proteins are boiled with acids they yield amino acids.

Plimmer: *The Chemical Constitution of the Proteins*, Longmans (1911).

### PROTEOSE SERIES

Proteins have limited solubilities and when treated with certain enzymes, thus lowering the molecular weight, the split-products usually have increased solubilities (exception in the action of rennin on casein). Proteoses are precipitated by ammonium sulfate. Zunz separated them into several fractions by ultra-filtration. Walters found proteoses in soil.

Walters: *Ind. Eng. Chem.* 7:860 (1915).

Zunz: *J. physiol. path. gén.* 12:884; *Bull. acad. roy. med. Belg.*, 3:426 (1923).

**Primary proteoses** are soluble in water, easily diffusible through membranes, non-coagulable by heat, precipitated by half saturation of their solutions with  $(\text{NH}_4)_2\text{SO}_4$ . They are also precipitated by ferric sulfate, trichloroacetic acid, picric acid, or nitric acid. They are divided into:

**Protoproteoses**, soluble in water and according to Zunz and György more toxic than the following group:

Zunz and György: *Arch. intern. Physiol.* 15:78 (1914).

**Heteroproteoses**, insoluble in water but soluble in dilute salt solution.

Wells and Osborne: *J. Chem. Soc.* 106:634 (1914); *Proc. Am. Soc. Biol. Chem.* (1914).

**Secondary proteoses** are precipitated by  $(\text{NH}_4)_2\text{SO}_4$  only at full saturation, and are not precipitated by  $\text{HNO}_3$ . They have lower molecular weights than primary proteoses.

Moraczewski and Lindner: *Biochem. Z.* 125:48 (1921).

Vaughan showed that alkaline hydrolysis would produce a proteose which is almost as toxic as a bacterial toxin, 10-15 mg. injected into the vein of a guinea-pig causing death.

Vaughan and Wheeler: *J. Infectious Diseases* 4:476 (1907).

Beginning with proteoses, proteins cause the phenomenon of anaphylaxis, i.e., one injection causing sensitization to injections several weeks later.

If to a proteose, which is made by the action of pepsin, more pepsin is added and the solution concentrated, synthesis occurs. The substance formed will have the same general solubility as the original protein but will not be identical; for example, **plastein** is obtained from the proteose formed when pepsin acts on albumin. To reverse the process, the solution is concentrated and more pepsin is added. Plastein is not albumin but has a higher molecular weight than the proteose.

Various proteoses have been recommended to patients, one which is called somatose being derived from muscle protein.

Wasteneys and Borsook: *J. Biol. Chem.* 62:1, 15 (1924); 62:675 (1925).

#### METAPROTEIN SERIES

The solubility of a protein is changed by alkali or acid. It is then called an acid or alkali metaprotein; there is an acid and an alkali metaprotein for every native protein. They are insoluble in neutral solution but soluble in weak acid or alkali.

**Syntonin** is an **acid metaprotein** derived from muscle protein.

Abderhalden and Sasaki: *Z. physiol. Chem.* 51:404 (1907).

McFarlane, Dunbar, Borsook, and Wasteneys: *J. Gen. Physiol.* 10:437 (1927).

**Alkali metaproteins** rotate the plane of polarized light more than the natural proteins from which they are formed although they are racemized by continued action of alkali.

Dakin and Dudley: *J. Biol. Chem.* 15:263 (1913); 13:357 (1912).

#### COAGULATED PROTEINS

Egg albumin is coagulated by boiling, but, as in the case of some protein substances which coagulate without boiling (casein), coagulation is influenced by the hydrogen-ion concentration, and occurs more easily at the isoelectric point. The egg white of a certain bird will not coagulate at 100° because it is too far on the

alkaline side of the isoelectric point. Certain chemists have claimed that coagulation involves splitting of protein. Coagulated egg white, as well as milk protein, is digested more rapidly than the uncoagulated. Serum albumin has a coagulation temperature of 67°.

Sørensen: Compt. rend. trav. lab. Carlsberg 15:1 (1925).

#### PROTAMIN SERIES

Protamins seem to be huge polypeptides (molecular weight about 2000). They have been found mainly in the heads of the spermatozoa of different animals, particularly fish. Kossel isolated several of these protamins. They are named from the fish from which they are obtained: **scombrin** (mackerel), **clupein** (herring), **salmin** (salmon), **sturin** (sturgeon), **percin** (perch), **esoxin** (*Esox lucius*), **cyprinin** (carp), **cyclopterin** (lump fish), and **crenilabrin** (cunner). They are very high in arginine and are highly basic, but not so basic as to be insoluble in ammonia. A few of them contain tyrosine. They are non-coagulable by heat and are soluble in acids. Protamins are not hydrolyzed by pepsin and not very rapidly by unactivated trypsin. By hydrolysis, complete recovery of amino acids has been obtained.

Protamins are hydrolyzed by protaminases (Waldschmidt-Leitz) which are carboxy-polypeptidases with special affinity for arginine (Grassman).

Miescher: Histochem. u. physiol. Arbeiten, 2, Leipzig (1897).

#### HISTON SERIES

Histons are similar to protamins (in fact, **sturin** approaches histons in composition and reaction to enzymes, not being split by trypsin), being even more basic (due to high content in hexone bases), and soluble in acid solution but insoluble in ammonia. The main difference is in their higher molecular weight (6000–10,000) and their content of a greater variety of amino acids, including arginine, histidine, lysine, tyrosine, and cystine. Sturin is similar in composition to histons. Histons occur in cell nuclei in combination with nucleic acid and are classified according to the animals and plants from which they are obtained. Besides being found in the nuclei of cells, they are found in the cytoplasm of erythrocytes. They are also found in the spermatozoa of some

fish (shad, cod). One histon of the erythrocytes is called **globin**. It is not free but is combined with hematin in hemoglobin.

Kossel: Protamins and Histons, Longmans (1914).

#### ALBUMIN SERIES

Albumins contain a much longer list of amino acids than histons but lack glycine. Many of them support the growth of young animals when forming the sole protein of the diet.

**Ovalbumin**, molecular weight 34,500: The white of egg contains mainly water and ovalbumin. The ovalbumin can be crystallized if purified to some extent. If the white of egg is diluted with distilled water a precipitate of globulin appears. If this is removed albumin and salts remain. If this is saturated with  $(\text{NH}_4)_2\text{SO}_4$  the egg albumin will precipitate. This can be dissolved in water and reprecipitated. After obtaining it fairly pure it will crystallize as sulfate. It can be recrystallized any number of times. This was done by Sørensen. The crystals of albumin sulfate are formed of 2 molecules albumin, 3 molecules  $\text{H}_2\text{SO}_4$ , and 830 water molecules. Egg albumin is split by pepsin but only slowly by trypsin; its isoelectric point is  $\text{pH}$  4.8.

It has yielded on hydrolysis 2.2% alanine, 2.5% valine, 10.7% leucine, 4.15% proline, 13.96% glutamic acid, 1.36% hydroxyglutamic acid, 6.07% aspartic acid, 5.1% phenylalanine, 4.21% tyrosine, 5.03% arginine, 6.41% lysine, 2.44% histidine, 1.33% cystine, 1.28% tryptophan, total 65.74%.

Calvery: J. Biol. Chem. 94:631 (1932).

Sørensen: Comptes rend. trav. lab. Carlsberg 12:1 (1917).

**Serum albumin**, molecular weight 45,000, has not been obtained in as pure a state as ovalbumin since the last trace of globulin cannot be removed. It is more resistant to trypsin-kinase than ovalbumin.

It is thought that serum albumin passes into the urine in albuminuria. The albumin in the urine has the same isoelectric point,  $\text{pH}$  4.8, as serum albumin. Cavett has made extensive analyses of urinary albumin by the Van Slyke nitrogen distribution method and racemization curves, and finds it similar to serum albumin.

Serum albumin acts as a buffer in the blood serum. The chief

buffer is bicarbonate, but if this is removed there remains considerable buffer action (fig. 51).

Sørensen: Proteins, Fleischmann Lab., 1925.

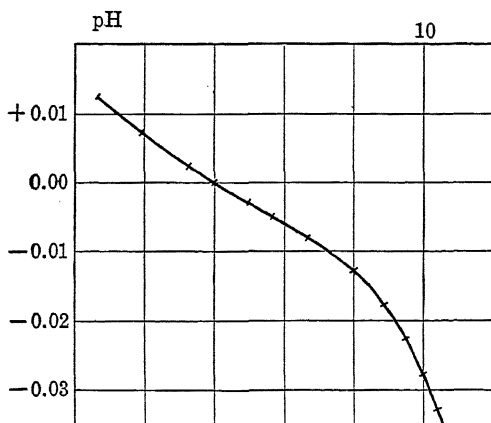


FIG. 51. Titration curve of carbonate-free blood plasma. Journal of Biological Chemistry.

**Lactalbumin** has not been completely purified. It has all the amino acids except glycine, judged by analysis and from feeding tests on growing rats. It contains 7% tryptophan and 2.6% methionine. Its isoelectric point is pH 4.55. It constitutes about 15% of the protein of cow's milk and probably 50% of the protein of human milk (Meigs and Marsh did not find such a difference between the proteins of cow's and human milk).

Meigs and Marsh: J. Biol. Chem. 14:147 (1913).

Osborne found in various plants a whole series of albumins named for the different plants (**leucosin**, **legumelin**, **phaselin**).

Osborne: The Vegetable Proteins, Longmans, New York (1924).

**Toxalbumins** agglutinate erythrocytes. **Ricin** from castor beans is very toxic. One large bean may be fatal. It is affected very slowly by digestive enzymes and hence retains its toxicity in the gut. The lethal dose is about 0.01 mg. per kg. rabbit. **Crotalin**, **cobra venom**, is a toxalbumin but is not as toxic if taken by mouth because it is digested by enzymes. The lethal dose by injection is about 0.08 mg. per kg. **Abrin** from *Abrus* (jumble beans) may be an albumose. **Robin** from *Robinia* (locust tree)

may be a nucleoprotein. **Sojaphasin** from soy beans is one of a group of vegetable proteins which agglutinate erythrocytes.

Carmichael: J. Pharmacol. 35:193, 223 (1929).

Hein: Compt. rend. soc. biol. 91:1223 (1924).

**Myogen** is a protein, probably an albumin, found in muscle. There is 4 times as much myogen as myosin in normal muscle.

Rona and Weber: Biochem. Z. 203:429 (1928).

**Pepsin** is a protein, probably an albumin.

Northrop dialyzed commercial pepsin until it precipitated (from acid solution), or precipitated it with  $MgSO_4$ . The precipitate was dissolved by warming the suspension to  $45^\circ$ . Crystals formed on cooling. Its proteolytic activity remained constant through 7 recrystallizations. The isoelectric point is pH 2.85. The percentage composition is: Total N 15.3, amino N 0.8, C 52.4, H 6.67, S 0.86, P 0.078, molecular weight about 40,000.

Northrop: Ergebnisse der Enzymforschung, 1:302, Leipzig (1932).

#### GLOBULIN SERIES

The albumins and globulins differ mainly in their solubility. Albumins are soluble in water and globulins are not. If water (8 or 10 times the volume of egg white) is mixed with egg white, the globulin will precipitate. Globulin is kept in solution in the egg by the dilute salt solution (about 1% salt, mostly NaCl but some  $NaHCO_3$ ).

The salting-out method is used in differentiating proteins. Various substances can be salted out from solution. Alcohol, air, or carbon dioxide may be salted out from water. Different salts differ in their effectiveness in salting out.  $K_2CO_3$  is the most effective in laboratory use for salting out alcohol. If one makes a mixture of equal parts of water and alcohol, and stirs in dry  $K_2CO_3$ , the alcohol will form a layer on top. There will be only about 5% of the alcohol left in the water and a small amount of water in the alcohol. Most of the  $K_2CO_3$  will remain in the water layer, as it is soluble in it but not in alcohol.

Ammonium sulfate is used in salting out egg albumin as it is the only abundant salt that is soluble enough to salt out albumins. Some of it combines with albumin to form albumin sulfate. The albumins are salted out by saturation with ammonium sulfate and

globulins by half-saturation, but the exact concentration depends on how near they are to their isoelectric points.

The globulins are of much higher molecular weight than the albumins, perhaps double. Since they are colloidal systems and a single molecule might constitute a colloidal particle, the size of the globulin particles should be larger; this perhaps accounts for the lesser solubility of globulin.

**Egg globulin** contains all the amino acids that have so far been found in proteins, and is said to contain 11% glucosamine. Proteins containing only small percentages of sugar groups are classed as simple proteins, and some investigators have thought that the sugar groups might be due to impurity.

Hertzman and Bradley: *J. Biol. Chem.* 61:275 (1924):

**Serum globulin** is resistant to pepsin and trypsin. Although it does not pass the nephritic kidney as easily as albumin since its molecular weight is 103,000, it is said to occur in many samples of albuminous urine. It has been separated into water-insoluble **euglobulin** and water-soluble **pseudoglobulin**.

Sjorgren: *J. Am. Chem. Soc.* 50:18 (1928).

**Fibrinogen** is a globulin which occurs in the blood. It is soluble in salt solution and in blood plasma. On coagulation, fibrin, a conjugated protein (see below) separates out.

Fibrinogen can be salted out of blood plasma that is prevented from coagulating.

Fibrinogen is formed in the liver and is reduced in liver disease.

Kaufman: *Z. ges. exptl. Med.* 62:165 (1928).

**Myosin** is found in muscle. It is said to arise from myogen, the change being said to be the cause of the clotting of muscle press juice. It is soluble in dilute salt solution, from which it may be salted out.

Wohlisch and Schriever: *Z. Biol.* 82:265 (1925).

**Thyroglobulin** occurs in the thyroid gland. Unless an animal obtains iodine, it cannot synthesize this globulin. The "colloid" of the thyroid gland is mainly thyroglobulin. No matter what kind of a thyroid gland an animal has (normal, embryonic, hyperplastic, adenomatous), if it is fed iodine, colloid will eventually be formed.

By alkaline hydrolysis of thyroglobulin, Kendall obtained

thyroxine. Alkali is used in hydrolysis as more is destroyed by acid.

Thyroglobulin increases the metabolic rate. It is classed as a pseudoglobulin as it is soluble in water. Cavett has spent much time in purifying it. His purest sample of hog thyroglobulin contained 0.69% iodine.

Cavett and Seljeskog: J. Biol. Chem. (Sci. Proc.) 100:xxvi (1933).

Hektoen and collaborators: J. Am. Med. Assoc. 84:114 (1925); 80:386 (1923); Am. J. Physiol. 71:548 (1925).

$\alpha$  and  $\beta$  **Crystallin** are the globulins of the crystalline lens of the eye. The name globulin was derived from the eye.

Guyer and Claus: Proc. Soc. Exptl. Biol. Med. 26:65 (1928).

**Bence-Jones protein**, molecular weight 24,500, found in urine of certain patients suffering from bone-marrow disease (multiple myeloma), coagulates at 50–58° and redissolves on boiling in the presence of salts. It is sometimes considered an albumose.

Bannick and Greene: Arch. Internal Med. 44:486 (1929).

**Pancreatic lipase** is considered to be a globulin by Glick and King. By drying lamb pancreas and extracting lipoids with acetone, it is made soluble in alkaline 10% NaCl solution. It is precipitated by saturating with  $MgSO_4$  at pH 4.5. By reprecipitation the lipase is concentrated 894 times.

Glick and King: J. Am. Chem. Soc. 55:2445 (1933).

**Pancreatic amylase** is considered a protein. It forms crystals.

Caldwell, Booker, and Sherman: Science 74:37 (1931).

**Trypsin**, C 50%, H 7.1%, N 15%, Cl 2.9%, S 1.1%, molecular weight 34,000. Pancreas press juice is extracted with 0.25 *N* HCl, filtered, made 0.4 saturated with ammonium sulfate, filtered, brought to 0.7 saturated with ammonium sulfate to precipitate the trypsin. It is reprecipitated repeatedly, and finally the precipitation is begun between 0.4 and 0.7 saturated  $(NH_4)_2SO_4$  and it crystallizes. At low salt concentration and pH 1–7 no loss of activity occurs on bringing to a boil and cooling, but it precipitates when salt is added to a hot solution. Northrop and Kunitz think it is denatured in hot solution but a reversal takes place by cooling if the salt concentration is low.

Northrop and Kunitz: J. Gen. Physiol. 16:267, 295 (1932).

**Chymotrypsin.** Northrop crystallized (elongated prisms) chymotrypsinogen from protein extracted from pancreas with  $M/8$   $H_2SO_4$  and precipitated at 0.7 saturated ammonium sulfate at pH 5. When 1 mg. of trypsin is added to 2 g. chymotrypsinogen dissolved in 400 cc. buffer solution at pH 7.6 at 5° for 24 hours, its proteolytic power increased 1000 times. Northrop called the protease formed "chymotrypsin" and precipitated it with 0.7 saturated ammonium sulfate. Its activity is one-third that of trypsin but digestion of casein is carried further.

Kunitz and Northrop: Science 78:558 (1933).

Several plant globulins have been shown to support growth when forming the sole protein of the diet.

Plant globulins are so numerous that only two groups may be considered here. First, globulins from seeds of legumes are higher in glutamic acid than animal globulins, as shown in the following table by Waldschmidt-Leitz:

Amino acid	Phase- olin	Leg- umin ( <i>Pisum</i> )	Vici- lin	Leg- umin ( <i>Vicia</i> )	Glyci- nin	Con- glutin	Jack bean urease
Glycine.....	0.6	0.4	0	0.4	1.0	0.8	
Alanine.....	1.8	2.1	0.5	1.2	—	2.5	
Valine.....	1.0	—	0.2	1.4	0.7	1.1	
Leucine.....	9.7	8.0	9.4	8.8	8.5	6.8	
Phenyla- lanine.....	3.3	3.8	3.8	2.9	3.9	3.1	
Tyrosine....	2.8	3.8	2.4	2.4	1.9	3.7	4.94
Asparatic acid.....	5.2	5.3	5.3	3.2	3.9	3.0	
Glutamic acid.....	14.5	17.0	21.3	18.3	19.5	23.0	
Arginine....	4.9	11.7	8.9	11.1	7.7	10.93	
Lysine.....	4.6	5.0	5.4	3.7	3.4	2.74	
Histidine....	2.6	1.7	2.2	2.9	2.1	2.51	
Proline.....	2.8	3.2	4.1	4.0	3.8	2.6	
Ammonia....	2.1	2.1	2.0	2.2	2.6	2.6	
Cystine.....	0.4	—	—	—	1.18	—	1.2
Tryptophan.	—	—	+	—	1.37	1.45	1.46

Waldschmidt-Leitz: Meyer and Jacobson's Org. Chem. 2(4, F) Berlin (1930).

**Urease** is obtained by Sumner by stirring 100 g. jack bean meal with 500 cc. 31.6% acetone and filtering in an ice box.

Urease crystallizes out in 12 hours, and crystals are washed in 32% acetone by centrifuging. They are redissolved in water,

and phosphate buffer (pH 6) is added slowly to induce crystallization. Their percentage composition is C 52, H 7.15, S 1.25, ash 2. The urease activity of the crystals is 700–1400 times as great as the meal or 133,000 units per gram. One unit will hydrolyze sufficient urea to form 1 mg. ammonia N in 5 minutes at 20°, pH 7. Ordinary distilled water contains copper or other metals that inactivate urease so that it should be dissolved in water from a glass or silica still.

Sumner: *Ergebnisse Enzymforschung*, 1:295, Leipzig (1932).

Globulins from oily seeds other than the above are high in arginine, as shown in the following table by Waldschmidt-Leitz:

Amino acid	Edestin ( <i>Cannabis sativa</i> )	Globulin ( <i>Ricinus communis</i> )	Excelsin ( <i>Bertholletia excelsa</i> )	Globulin ( <i>Cucurbita pepo</i> )	Amandin ( <i>Prunus amygdalus</i> )	Arachin ( <i>Arachis hypogaea</i> )
Glycine.....	3.8	—	0.6	0.6	0.5	—
Alanine.....	3.6	—	2.3	1.9	1.4	—
Valine.....	6.2	—	1.5	0.7	0.2	—
Leucine.....	14.5	—	8.7	7.3	4.5	—
Phenylalanine	2.4	—	3.6	3.3	2.5	—
Tyrosine.....	2.1	—	3.0	3.1	1.1	—
Asparatic acid	4.5	—	3.9	4.5	5.5	—
Glutamic acid	14.5	14.5	12.9	13.4	23.1	—
Arginine.....	15.8	13.2	14.3	14.4	12.2	11.2
Lysine.....	3.9	1.5	1.6	2.0	0.7	5.1
Histidine.....	4.0	2.7	1.5	2.6	1.9	2.1
Proline.....	1.7	—	3.7	2.8	2.4	—
Ammonia.....	2.3	2.4	1.8	1.6	3.7	1.9
Cystine.....	0.3	—	0.525	—	0.43	—
Methionine...	2.1	—	—	—	—	0.5
Tryptophan...	+	—	+	—	+	—
M. W.	2900					

Waldschmidt-Leitz: Meyer and Jacobson's *Org. Chem.* 2(4, F) Berlin (1930).

Protein may be extracted from *wheat flour* by neutral salt solutions and has been called globulin on account of its solubility. Gortner has shown that the quantity of protein extracted depends on the neutral salt used. Thus NaI extracts far more protein than NaCl. Gortner concludes that salts "peptize" the protein and in their efficiency follow Hofmeister's series.

Soy beans, although legumes, in composition resemble the oily seeds in the above table, and are often used for diabetics, as they contain very little available carbohydrate. Soy-bean flour was

introduced into Austria by Berczeller for economical reasons. The *soy-bean proteins* are better proteins than other bean proteins, as they contain all the amino acids, whereas the other bean proteins must be supplemented by meat or other expensive proteins.

Adolph and Kiang: Nat. Med. J. China, 6:40 (1920).

**Conglutin**, after the lupine-toxin was removed, was eaten in Europe during the war.

Bokorony: Biochem. Z. 100:100 (1919).

Potatoes contain **tuberin** of high biological value, and also a toxin (solanine), which is, however, mainly confined to green parts.

Kon: Biochem. J. 22:261 (1928).

#### PROLAMIN OR GLIADIN SERIES

Prolamins are high in proline and soluble in 70% alcohol, and are found in cereal grains (oats, rye, wheat, Indian corn; barley, and other cereals). It is not known whether prolamins of the same name are the same when obtained from different cereals. Gortner purified them and Wells found them different serologically.

**Gliadin** is the chief protein of wheat gluten, the protein portion of the endosperm (fig. 52). If wheat flour is washed with water, the starch is removed and gluten remains, which contains gliadin. Gliadin, molecular weight 20,700, is about 40% glutamic acid, 15% proline, 6% leucine, 4% valine, 3% arginine, 3% phenylalanine, 2% tyrosine, 2% alanine, 1.5% histidine, 2% methionine, and is therefore adequate in these amino acids. It does not allow normal growth of white rats in whose diet it is the sole protein. This failure may be attributed to its low content in cystine (0.45%), lysine (0.63%), and tryptophan (1%). When rats receive 80% of their diet in the form of gliadin, some growth occurs.

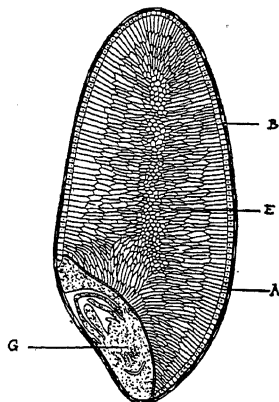


FIG. 52. Wheat grain: A, aleuron; B, bran; E, endosperm; G, germ. Committee on Accessory Food Factors, Sp. Rpt. No. 38.

Gliadin is the chief protein of bread as there is only two-thirds as much glutenin as gliadin in bread (wheat).

In Japan, glutamic acid is made by hydrolyzing wheat gluten, which is about 60% gliadin and 40% glutenin.

The prolamins of rye, durum, einkorn, emmer, and spelt are also called "gliadin" although they may differ from that of wheat. Prolamin is necessary to add the right consistency to bread, and its absence from rice is the reason for the absence of rice-bread, although crisp rice cakes are made in Japan.

Jones and Wilson: *Cereal Chem.* 5:437 (1928).

**Hordein**, from barley, is similar to gliadin in amino acids.

Nakamura: *J. Fac. Ag. Hokkaido Imp. U.*, 23:29 (1928).

**Zein**, from Indian corn, is the most deficient prolamins, containing no tryptophan, cystine, or lysine. It contains 2.4% methionine. If 2% of each of these amino acids is added to the zein, it becomes a good protein. Zein forms about 70% of corn gluten. It is very little affected by gastric digestion.

All prolamins are deficient in some of the essential amino acids. For this reason one could not live on bread alone, although it does give the required number of calories.

Hirsh: *Biochem. Z.* 198:379 (1928).

#### GLUTELIN (GLUTENIN) SERIES

Glutelins are contained in gluten (although there is less in corn gluten) and are soluble in acid and alkali and insoluble in water or alcohol. They contain a better balance of amino acids than prolamins and may support growth (Osborne and Mendel).

**Glutenin**, a glutelin from wheat, forms about 40% of wheat gluten. Blish has separated  $\alpha$ -glutenin from  $\beta$ -glutenin, which is more soluble.

Blish: *Cereal Chem.* 2:127 (1925); *J. A. Off. Ag. Chem.*, 9:417 (1926); 11:475 (1928).

**Oryzenin**, a glutelin from rice, is its chief protein.

Sahashi: *Biochem. Z.* 189:208 (1927).

#### SCLEROPROTEIN SERIES

These proteins are found in the skeletal structures, which include hair, nails, connective tissue, and bones. In bone, the scleroprotein matrix is stiffened by a crystal-lattice containing

$\text{CaX}[\text{Ca}_3(\text{PO}_4)_2]_3$ , where  $X$  may be  $\text{Cl}$ ,  $\text{F}$ ,  $\text{OH}$ , or  $\frac{1}{2} \text{CO}_3$ . Most scleroproteins are soluble in fairly strong alkali but may be altered by it. They are insoluble in all other solutions. They contain diketopiperazine rings, and most of them are not split by digestive enzymes. Some animals accumulate balls of hair in the stomach; others vomit to get rid of feathers.

**Keratin**, from the cuticle of the skin, nails, and hair, protects the skin from ultra-violet rays and becomes thicker on exposure to sun. It is not the melanin of sunburn that protects from ultra-violet rays but keratin. Powdered keratin and lanolin would be a good protection against sunburn. Metal in contact with the skin blackens owing to loosely combined sulfur. Keratin contains nearly 15% cystine. It is not digested by trypsin or pepsin and is insoluble in dilute acids, alkalies, and all organic solvents. The molecular ratios of histidine, lysine, and arginine are approximately 1:4:12.

Merrill and Fleming: *J. Am. Leather Chem. Assoc.* 22:139 (1927).

**Neurokeratin** is found in nerve tissue.

Nelson: *J. Am. Chem. Soc.* 38:2558 (1916).

**Ovokeratin** is found in eggshell lining.

Abderhalden and Ebstein: *Z. physiol. Chem.* 48:530, 535 (1905-6).

**Koilin** is found in the lining of the gizzard of birds.

Knaffl-Lenz: *Z. physiol. Chem.* 52:472 (1907).

**Elastin**, from yellow elastic fibers, differs from other scleroproteins in being digestible. A convenient source is the ligamentum nuchae.

Clark: *Proc. Nat. Acad. Sci.* 14:526 (1928).

**Collagen**, from white fibers, seems not to be digestible in its natural state by enzymes and is not attacked by trypsin.  $\text{HCl}$  in the stomach changes it to acid metaprotein which is digested by pepsin. The "bating of leather" with trypsin removes elastin but leaves collagen unharmed. When boiled it is changed to gelatin, which is digestible. It is combined with tannin or chromium in the tanning of leather. It is sometimes called **ossein** in bone.

Sommer and Rose: *J. Biol. Chem.* 80:157 (1928).

**Gelatin** may be obtained from bone by steaming. It is not a pure protein. Glue is gelatin in the sol stage, being kept in this

state by impurities. Acid will keep it in this state by preventing it from reaching the isoelectric point. Gelatin contains 17% glycine but no tyrosine, valine, or tryptophan, and being deficient in serine and cystine is, therefore, a poor protein. As glycine can be synthesized in the body and when eaten it changes over to sugar, the more glycine present the poorer the protein. Rats fed on a diet containing 35% gelatin plus additional cystine, tyrosine, and tryptophan grew at subnormal rates. When 11 supplementary amino acids were added to gelatin, growth was subnormal. Gelatin is used as a colloid to give consistency to ice cream and other foods.

Johlin: J. Biol. Chem. 86:231 (1930).

**Reticulin** is a scleroprotein of reticular tissue.

Siegfried: J. Physiol. 28:319 (1893).

**Osseoalbuminoid** is found in bone.

Hawk and Gies: Am. J. Physiol. 7:340 (1902).

**Eye albuminoids** form about 50% of the protein of the crystalline lens.

Dodd, Floszner, and Kulscher: Z. Immunitäts. 59:310 (1927).

**Fibroin**, from silk, is affected by ultra-violet light so as to reduce the tensile strength of silk fibers. It is a good protection against sunburn. It contains glycylalanine and alanyl-serine diketopiperazines.

Herzog: Helv. Chim. Acta 11:529 (1928).

**Ichthylepidin**, from fish scales, forms 20% of their organic compounds.

Abderhalden and Voitinovici: Z. physiol. Chem. 52:368 (1907).

**Conchiolin**, from oyster shell.

Story and Andratsche: Z. physiol. chem. 148:83 (1925).

**Gorgonin** containing diiodotyrosine — from gorgonian skeleton (sea fan).

Oswald: Z. physiol. Chem. 75:353 (1911).

**Spongin** containing diiodotyrosine — from bath-sponge skeleton. It has been used as a preventive or for the cure of goiter. The ash has been used also, as the reducing action of the carbon removes the iodine and changes it to iodide, which remains in

the ash in combination with mineral bases that are present as impurities.

Wheeler and Mendel: *J. Biol. Chem.* 7:1 (1910).

### CONJUGATED PROTEINS

A conjugated protein is a simple protein conjugated with some entirely different chemical molecule, known as the prosthetic group. The presence of a small percentage of glucosamine has not led biochemists to place a protein in this group, and the British biochemists omit phosphoglobulins from this group.

### CHROMOPROTEIN SERIES

The prosthetic group (group combined with a simple protein) in chromoproteins is colored. Globin, a simple protein, combines with 4% of "hematin," a prosthetic group, to form hemoglobin. Most preparations called "hematin" contain protein, however. The word "acid hematin" is used in spectroscopy, but the word "hemin," that was formerly defined as the chloride of hematin, is used more frequently in analytical work.

**Hemoglobin** (reduced hemoglobin) is the red coloring matter of blood, its spectrum showing an absorption band with center at 5590 Å. The protein component is globin, a histone. The prosthetic group, an iron-containing tetrapyrrol base, is described under nitrogenous bases. Anson and Mirsky synthesized hemoglobin from reduced "heme" and globin at pH 9.

Anson and Mirsky: *J. Gen. Physiol.* 12:273, 285 (1928).

**Oxyhemoglobin**, formed by the union of one molecule of oxygen with one molecule of hemoglobin, has two absorption bands in its spectrum centering at 5417 and 5775 Å. This oxygen may be removed by the air pump. It will crystallize, the crystalline form being different for different animals but the molecular weight about constant at (16,669)<sub>4</sub>.

The chief function of oxyhemoglobin is the carrying of oxygen from the lungs to the tissues. The concentration in the blood is given under iron (which see); in the adult it averages about 16%. (The corpuscles contain about 40% and the blood contains about 40% corpuscles.) Since the molecular weight is about 16,000 and 1 liter of blood contains about 160 g. or 0.01 mol, 1 liter of blood will carry about 224 cc. of oxygen. In other words, there is

about as much oxygen in oxygenated blood as in an equal volume of atmospheric air at 0° and 760 mm. mercury.

Arterial blood has an oxygen tension of about 100 mm. mercury, and at this tension about 2%  $\text{HbO}_2$  dissociates into hemoglobin and oxygen, leaving the hemoglobin about 98% saturated with oxygen. In very vascular, resting tissue with rapid circulation, the oxygen tension sinks to about 50 mm. mercury, and with no  $\text{CO}_2$ -production, only 14% dissociates, leaving the hemoglobin about 86% saturated with oxygen, thus giving up 7% of its oxygen to the tissues or liberating about 15 cc. oxygen per liter of blood. In case of more rapid utilization of oxygen in the tissues, its tension may sink until as a limit all of the oxygen is removed from the blood.

$\text{CO}_2$  drives oxygen out of the blood (this may be due to change of pH). At 50 mm. mercury, oxygen tension, and 20 mm. mercury  $\text{CO}_2$ -tension, 7% of  $\text{HbO}_2$  dissociates, and at 40 mm. mercury  $\text{CO}_2$ -tension 14% dissociates, therefore the  $\text{CO}_2$  produced by the tissues aids in driving the oxygen out of the blood whence it passes to the tissues.

The rôle of hemoglobin in the  $\text{CO}_2$ -carrying capacity of the blood was given above under the heading  $\text{CO}_2$  and is due to the fact that  $K_A$  for Hb is  $4.9 \times 10^{-8}$  and for  $\text{HbO}_2$  is  $6.9 \times 10^{-8}$ .

Barcroft: Respiratory Function of the Blood, Cambridge (1928).

**Carbonyl hemoglobin** (absorption bands centering at 5420 and 5700 Å) is hemoglobin combined with carbon monoxide. More than 50% of the hemoglobin of the blood is changed to carbonyl hemoglobin in cases of death from carbon monoxide poisoning. The blood holds on to carbon monoxide 210 times more strongly than to oxygen and will not carry oxygen when carbon monoxide is present in the air in significant amounts. Breathing air containing 0.2% carbon monoxide for 5 hours may cause death. This is a reversible reaction, and the blood may be rid of carbon monoxide by breathing air or oxygen. The blood can carry as much pure oxygen as ordinary air, and this capacity is greater than ordinary needs. A trace of carbon monoxide in the blood is of no significance; the normal is about 1% of the hemoglobin as carbonyl hemoglobin (Gettler).

As tannin precipitates hemoglobin but does not precipitate carbonyl hemoglobin (which remains a cherry red solution), it is used in determining the amount of carbonyl hemoglobin.

Carbonyl hemoglobin is decomposed by light of the same wavelength as its absorption spectrum; hence, its decomposition may be used to determine its presence in turbid solutions to which the spectroscope is not adapted; or more exactly, this method is used to determine hemoglobin since the carbon monoxide is measured after the decomposition of COHb.

Gettler and Mattice: J. Am. Med. Assoc. 100:92 (1933).

Van Slyke and Hiller: J. Biol. Chem. 84:205 (1929).

Butchers sometimes paint their meat with nitrate, which changes the hemoglobin to **nitric oxide hemoglobin** which is bright red as in hams, bacon, and corned beef. It has the same spectrum as oxyhemoglobin but is not reduced to hemoglobin on storage.

Oppenheimer: Handbuch d. Biochemie d. Menschen u. d. Tiere 1:654 (1910).

**Sulf-hemoglobin** arises from the action of  $H_2S$  and has an absorption band centering at 6175 Å.

Clarke and Hurtley: J. Physiol. 36:62 (1907).

**Cyanhemoglobin** arises from the action of HCN on hemoglobin and has an absorption band centering at 5495 Å.

**Methemoglobin** (absorption band centering at 6630 Å in acid solution; in neutral solution 4 bands: 5000, 5400, 5760, and the strongest at 6250 Å) is hemoglobin combined with *oxygen which cannot be removed by the air pump* but only by some chemical reducing substance. It is produced by aniline, paint poisons, and many oxidizing agents and other substances. It is excreted by the kidney with consequent loss of hemoglobin from the blood. It may be made synthetically from hematin and globin (Hill and Holden). When reduced it forms hemoglobin which by combination with oxygen forms oxyhemoglobin. Methemoglobin contains oxygen but in different combination with the iron, which is  $Fe^{++}$  in oxyhemoglobin and  $Fe^{+++}$  in methemoglobin.

Hill and Holden: J. Physiol. 61:22 (1926).

**Hemocyanin** is a colored protein containing 0.28% copper and occurring in the blood of molluscs and crustacea.

Cohn, Hendry and Prentiss: J. Biol. Chem. 63:1722 (1925).

**Oxyhemocyanin** gives up oxygen in a vacuum. In alkaline solution the absorption band is at 5760 Å, but varies with the species of animal from which derived.

Redfield, Collidge and Hurd: J. Biol. Chem. 69:475 (1926):

**NO-hemocyanin** forms green crystals.

Dhere and Schneider: J. physiol. path. gén. 20:1 (1922).

**Phycoerythrin** is a colored protein of red seaweed having three absorption bands in the green region of the spectrum.

Kitasato: Acta. Phytochim. 2:75 (1925).

**Phycocyanin** is a colored protein of blue-green algae having one or two absorption bands in the orange-red region of the spectrum. Other pigments are present, and the term "blue-green" may seem anomalous to the unaided eye.

Rodia: Atti acad. Lincei, 1:188 (1925).

#### GLYCOPROTEIN SERIES

A glycoprotein contains a simple protein conjugated with a carbohydrate complex. Glycoproteins contain two types of prosthetic groups: the mucoitin sulfuric acid group and chondroitin sulfuric acid group. These proteins are found in the form of the mucins in saliva and the trachea, and mucoids in connective tissue, skin of amphibians, and frog's egg jelly.

**Salivary mucin** contains mucoitin sulfuric acid and has an isoelectric zone, pH 1.5-4. Outside this zone it is glue-like and hence Gies advocated lemon juice or vinegar for washing it away from the teeth. Peptic digestion of mucin produces proteoses and peptones conjugated to the carbohydrate complex. Saliva contains 0.26% mucin.

Polson: Brit. J. Exptl. Path. 8:205 (1927).

**Bird's nest mucin** is the salivary mucin of the swift (*Collocalia*) of which it builds its nest in the limestone caves of Borneo. It is eaten by Chinese as the calcium salt. It contains 18% carbohydrate, 5.6% tyrosine, 1.4% tryptophan, 2.4% cystine, 2.7% histidine, 2.7% arginine. It is not digested by pepsin or trypsin.

Heiduschka and Graefe: Biochem. Z. 260:406 (1933).

**Gastric mucin** contains mucoitin sulfuric acid and is given to patients with gastric ulcer to bind the HCl of the gastric juice which irritates the ulcer. It is not digested by pepsin.

Webster: Trans. Roy. Soc. Can. 24:199 (1930).

## CHONDROPROTEINS

They contain chondroitin sulfuric acid and twice as much sulfur (about 2.4%) as mucin contains.

**Chondromucoid** is insoluble in water but soluble in dilute alkali.

Mörner: *Z. physiol. Chem.* 18:60, 213, 233 (1894).

**Tendon mucoid** is slightly soluble in 0.1% HCl.

Richards and Gies: *Am. J. Physiol.* 7:93 (1902).

## PHOSPHO-PROTEIN SERIES

This series contains phosphoric acid as the prosthetic group and excludes the lecithoproteins and nucleoproteins in which the prosthetic group consists of phosphoric acid, united in a complicated organic structure.

**Casein**, molecular weight 192,000, the characteristic protein in milk of all mammals, is not heat-coagulable. Cow's milk contains 3-4% and human milk contains 0.5-1.5% casein. Casein is acted upon by an enzyme, rennin, and is converted into **paracasein**, which coagulates with the aid of  $\text{Ca}^{++}$ . As a large portion of a calf's diet is milk, rennin can be found in the stomach-lining, and from this rennet-tablets are made. A tablet dissolved in a cup of warm milk causes coagulation in a short time. Casein contains all the necessary amino acids in good proportion for nutrition (except that the cystine content is low) and has, therefore, a very high nutritive value, but not so high as lactalbumin or the mixed proteins of skeletal muscle.

Casein is precipitated by bringing skim milk to the isoelectric point of casein, pH 4.7. If this is done with rapid stirring (L. L. Van Slyke) a fine precipitate, that may be washed in the centrifuge, is obtained, but if stirred very little, grain-curd casein is produced which may be washed by filtering through cheesecloth (W. M. Clark).

Van Slyke and Bosworth: *J. Biol. Chem.* 14:227 (1913); 19:67 (1914).

**Vitellin** from egg yolk is probably (as it exists in the egg) a phosphoprotein combined with lecithin (see lecithoproteins).

Waldschmidt-Leitz and Kunster: *Z. physiol. Chem.* 171:7 (1927).

## NUCLEOPROTEIN SERIES

In obtaining nucleoproteins the tissue is extracted with alcohol to remove lecithin and then extracted with dilute acetic acid to remove inorganic phosphoric acid. The nucleoprotein may then be extracted with dilute alkali.

Nucleoproteins occur in cell nuclei, and have been studied in thymus, spleen, pancreas, thyroid, liver, muscle, adrenal, mammary and submaxillary gland, gastric, lymphatic and sterile pus cells, all these having relatively large or abundant nuclei. Nucleoproteins are found in human erythrocytes which do not have nuclei, as well as hen erythrocytes which do. Nucleoproteins are also extracted from bacteria, mold, and yeast.

The best sources of nucleoproteins are the heads of spermatozoa. Fish testes are ground up, washed with salt solution and strained, and when free from pieces of tissue the suspension is centrifuged, the heads of the spermatozoa containing the nucleoprotein, being heavy, are torn away from the tails.

**$\alpha$ -Pancreas nucleoprotein** precipitated at its isoelectric point carries down 50% of the tryptic activity.

Michaelis and Davidsohn: *Biochem. Z.* 30:481 (1911).

**Thyroid nucleoprotein** is extracted with thyroglobulin and is difficult to separate from it.

Grobly: *Mitt. Grenzg. Med. Chir.* 30:403 (1918).

**Liver nucleoprotein** is said to have an antithrombin action, but this may be due to heparin carried down with it.

Larsell, Jones, Phelps and Nohes: *J. Am. Med. Assoc.* 90:75 (1928).

**Salmon sperm nucleoprotein** constitutes almost the entire sperm head and is said to carry the hereditary characters from the male. The lethal intravenous dose is 15-18 mg. per kg.

Mathews: *Z. physiol. Chem.* 23:299 (1897).

Bacterial nucleoproteins are antigenic.

Nucleoproteins dissolve in dilute salt solutions, but in the stomach they split into protein and nuclein, which precipitates.

In the intestine (which contains a nucleinase) the nuclein is split into protein and nucleic acid (tetranucleotide).

Nucleotidases in the intestine split the nucleotides into nucleo-

sides and phosphoric acid. It is said that pyrimidine nucleotides are split only in the intestinal wall.

Purine nucleosides are split in the intestinal wall, but enzymes for splitting pyrimidine nucleosides have recently been found by Deutsch in the red bone marrow.

Deutsch and Laser: *Z. physiol. Chem.* 186:1 (1929).

Nord and Weidenhagen: *Ergebnisse der Enzymforschung*, Leipzig (1932).

### LECITHOPROTEIN SERIES

The lecithoproteins contain lecithin as a prosthetic group and some other yolk proteins.

**Vitellin** probably belongs in this group. One of these, batrachio-lin, occurring as crystals in frog's eggs, contains 6% lecithin.

McClendon: *Am. J. Physiol.* 25:195 (1909).

**Fibrin** is probably the most important "lecithoprotein" or cephaloprotein as it forms blood clot which stops hemorrhage. There are two mechanisms of coagulation of blood, according to Mills. There are two types of fibrin, "tissue fibrinogen fibrin" and "thrombin fibrin." The fibrin in either case is a cephalin-protein compound. The clot may be formed in either of two ways: (1) Tissue fibrinogen fibrin is formed from union of tissue fibrinogen and blood fibrinogen in the presence of calcium salts in the blood. This form of fibrin consists of tissue protein, cephalin, calcium, and blood fibrinogen. (2) As thrombin does not exist in the blood, it must first be formed. Prothrombin does exist in the blood, probably in the blood platelets and other cells; and when it combines with cephalin and calcium, which are also present in the blood, thrombin is formed. Thrombin then combines with blood fibrinogen in the blood to form thrombin fibrin which contains platelet protein, calcium, cephalin, and blood fibrinogen.

If calcium is not present, clotting will not take place. Therefore, in cases where blood clotting is not wanted, for instance, when blood is drawn for analysis, the calcium may be precipitated as an oxalate by the addition of 0.1% potassium oxalate.

Mills has made a tissue extract from lung tissue which promotes clotting, and it has been used in hemophiliacs (people whose blood does not clot).

Mills: *Chinese J. Physiol.* 1:3 (1927).

## DIVISION 5

### NITROGENOUS BASES

(INCLUDING PUTREFACTIVE PRODUCTS, PTOMAINES)

The nitrogenous bases are called alkaloids when they come from plants, and ptomaines when they come from animals. Some of them in the body are called hormones.

#### AMINE SERIES

**Ammonia**,  $\text{NH}_3$ , is in very low concentration in blood (0.1 mg. per 100 cc.). It is a poisonous substance and is detoxicated by being changed to urea in the liver. The kidney hydrolyzes some of the urea by means of urease but excretes most of the  $\text{NH}_3$  as soon as it is formed. It constitutes about 4–12% of the nitrogen in the urine, and a normal individual excretes about 0.4–0.7 g. of ammonia per day.

The chief buffer of the blood is sodium bicarbonate. Acids are excreted as sodium or potassium salts; but if there is a shortage of sodium, some of the urea in the kidney is hydrolyzed; and the acids are excreted as ammonium salts; and the sodium is retained in the body.

If  $\text{NH}_4\text{Cl}$  is eaten, the ammonia is changed to urea; and there is an increase in the acidity of the urine due to the  $\text{HCl}$  excreted. Some of the urea is hydrolyzed in the kidney, and there is an increase in the  $\text{NH}_3$  and titrable acidity of the urine.

Ammonia is said to arise during muscular contraction by the conversion of adenylic acid into inosinic acid.

Scopoli discovered sal ammoniac in gastric juice. Although blood contains very small amounts of  $\text{NH}_3$ , gastric mucosa contains 40–50 mg. per 100 g. Mathews pictures the gastric secretion as (1) the secretion of  $\text{NH}_4\text{Cl}$  and (2) the re-absorption of  $\text{NH}_3$  leaving  $\text{HCl}$  in the stomach.

Benedict and collaborators: *J. Biol. Chem.* 48:463 (1921); 51:183 (1922); 60:491 (1924); 69:381, 395 (1926).

Mann: *J. Am. Med. Assoc.* 85:1472 (1925).

**Methyl amine**,  $\text{CH}_3\text{NH}_2$ , occurs in some plants and is sometimes found in the urine, probably arising from glycine in the gut.

Hiruma: Arch. ges. Physiol. 200:497 (1924).

**Dimethyl amine**,  $(\text{CH}_3)_2\text{NH}$ , is formed from choline and occurs in herring-brine, owing to putrefaction.

Orient: Biochem. Z. 132:352 (1922).

**Trimethyl amine**,  $(\text{CH}_3)_3\text{N}$ , is formed from choline and occurs in some plants.

Langley: J. Biol. Chem. 84:561 (1929).

These amines react with water to form bases in the same manner as ammonia. The basicity increases with increased substitution of hydrogen atoms. Ethyl amine arises by decarboxylation of alanine.

Petit and Perlis: Comp. rend. soc. biol. 94:978 (1926).

**Isobutylamine**,  $(\text{CH}_3)_2\text{CHCH}_2\text{NH}_2$ , arises by decarboxylation of valine in the intestine.

Hanzlik: J. Pharmacol. 17:327 (1921).

**Isoamylamine**,  $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{NH}_2$ , occurs as a product of decarboxylation of leucine in the intestine.

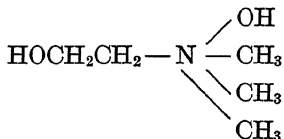
Nakamura: Tohoku J. Exptl. Med. 6:367 (1925).

The following bases occur in phospholipins:

**Hydroxyethyl amine**,  $\text{HOCH}_2\text{CH}_2\text{NH}_2$ , occurs as the base of cephalin. It is formed by decarboxylation of serine (Nord).

Trusler: Ind. Eng. Chem. 21:685 (1929).

**Choline**, hydroxyethyl-trimethyl-ammonium-hydroxide,



is the base of lecithin. Choline and acetyl choline have been claimed to increase intestinal peristalsis and have been called the hormones of intestinal tone. Choline is said to increase in sweat before menstruation. The lethal dose is 35 mg. per kg. It decreases blood pressure. Choline and acetylcholine delay the coagulation of the blood.

Viale: Endocrinology 13:296 (1929).

**Acetylcholine**,  $(\text{CH}_3)_3 - \text{N} - \text{CH}_2\text{CH}_2\text{O} - \text{CCH}_3$ , was first found

$$\begin{array}{ccc} & | & || \\ & \text{OH} & \text{O} \end{array}$$

in ergot (rye smut). It is said to occur in traces as a constituent of lecithin. Its stimulating action on the parasympathetic nervous system (increasing intestinal peristalsis, lowering blood pressure) is said to be 100,000 times that of choline. It is rapidly destroyed (hydrolyzed) in the blood.

Dale and Dudley: *Z. physiol. Chem.* 198:85 (1931).

Hunt: *Am. J. Physiol.* 45:197 (1918).

**Muscarine**,  $\text{O}:\text{CH}\cdot\text{CH}_2\text{N}(\text{CH}_3)_3\text{OH}$ , occurs in poisonous mushrooms and is said to arise by putrefaction of choline. It lowers blood pressure. The lethal dose is 3 mg. subcutaneously.

Baur: *Arch. exptl. Path. Pharmacol.* 134:49 (1928).

**Neurine**,  $\text{CH}_2:\text{CHN}(\text{CH}_3)_3\text{OH}$ , is derived from choline by putrefaction in the gut. It increases blood pressure. The lethal dose is 50 mg. per kg.

Houssay and Molinelli: *Compt. rend. soc. biol.* 98:172 (1928).

**Sphingosine**,  $\text{C}_{12}\text{H}_{25}\text{CH}:\text{CHCHOH}\cdot\text{CHOHCH}_2\text{NH}_2$ , occurs in sphingomyelin and phrenosin. It forms a purple color with  $\text{CuSO}_4$  solution on the addition of a little sugar.

Levene and Haller: *J. Biol. Chem.* 63:669 (1925).

### UREA SERIES

**Urea**,  $\text{NH}_2\text{CONH}_2$ , is formed largely in the liver. It accounts for 80% of the total nitrogen in urine, and there are normally 11–25 mg. per 100 cc. blood. Not only in man but also in amphibia and most fish, urea forms the chief end-product of protein metabolism. Urea is hygroscopic, and its excretion is accompanied by the excretion of considerable water. The above animals require water either as such or in their food.

Reptiles and birds are, however, desert organisms, some reptiles and birds spending their whole life in the desert. The migratory birds are deprived of water only on their migratory flights, in which case they have not the opportunity of drinking during many hundred miles of travel. Associated with this lack of water is the replacement of the urea in the urine by uric acid, a substance which is not hygroscopic and which is excreted in the dry form.

A normal adult eats about 100 g. of protein a day and excretes about 16 g. of nitrogen or about 30 g. of urea. The non-urea nitrogenous constituents of the urine are more constant than urea so that, on a very low nitrogenous output, the percentage of nitrogen as urea may fall to 50.

In the formation of urea from ammonia, it is supposed that ammonium carbamate is an intermediate product since it is found in the blood and urine of dogs with an Eck-fistula (an artificial passageway diverting the portal blood so that it passes directly back to the heart instead of through the liver, in this way putting the liver out of the general circulation). A still more drastic operation is the total removal of the liver as has been done by Mann at Rochester, Minnesota.

After this operation the urine and blood contained much less urea than normally. After removal of both the liver and kidneys of a dog, the urea content of the blood remained constant showing that no more was formed. It seems possible that urea is formed in other tissues, since Fiske and Sumner found an accumulation of urea in cats whose abdominal viscera were tied off, on injecting amino acids intravenously. Rabinowitch observed absence of urea in human blood in a case of yellow atrophy of the liver.

Uremia is a condition in which urea is retained in the blood. Folin (see table p. 262) has shown that when one form of non-protein nitrogen is retained, the other forms are also retained. In a case of nephritis, the following number of milligrams were in 100 cc. blood: Urea nitrogen 193; creatinine 16; uric acid 12; non-protein nitrogen 258. As urea nitrogen increases, the others also increase. The non-protein nitrogen is equivalent to the sum of the other nitrogens. The residual nitrogen is the nitrogen broken off from protein during analysis and is zero in unclaked blood.

In cases of nephritis it is necessary to know only the non-protein nitrogen as the sum of the other nitrogens is equal to it.

Bollman, Mann, and Magath: *Am. J. Physiol.* 69:371 (1924).

Fiske and Sumner: *J. Biol. Chem.* 18:285 (1914).

Folin: *Physiol. Rev.* 2:460 (1922).

Rabinowitch: *J. Biol. Chem.* 83:333 (1929).

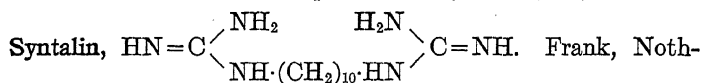
**Guanidine**,  $\text{NH}=\text{C}(\text{NH}_2)_2$ , is a constituent of arginine but occurs free in the blood (0.02–0.2 mg. per 100 cc.). It has been studied considerably, and it has been thought that the parathyroid

Per 100 cc. whole blood							Per 100 cc. plasma							Per 100 cc. corpuscles							Corpuscles
Amino acid N	Urea N	Creatinine	Uric acid	Undetermined N	Total non-protein N		Amino acid N	Urea N	Creatinine	Uric acid	Undetermined N	Total non-protein N		Amino acid N	Urea N	Creatinine	Uric acid	Undetermined N	Total non-protein N		
mg.	mg.	mg.	mg.	mg.	mg.		mg.	mg.	mg.	mg.	mg.	mg.		mg.	mg.	mg.	mg.	mg.	mg.		
5.0	13	1.8	3.7	19	39	4.3	13	1.8	3.7	20	39	5.9	13	1.8	3.7	18	39	45		vol.	per cent
6.4	49	2.0	4.5	7.4	65	5.8	47	2.3	6.4	18	74	7.2	52	1.5	1.6			52?		40	
4.5	49	3.0	4.7	22	78	3.5	52	3.0	6.1	18	77	7.4	41	3.0	0.9	31	81			27	
5.8	60	7.7	4.8	37	107	5.4	71	8.1	6.8	21	103	7.2	26	6.5	0	86	122			23	
7.6	91	7.2	6.4	41	144	6.2	109	8.1	9.3	23	144	11.8	38	4.4	0	93	144			25	
8.4	174	12.9	13.6	75	267	7.5	214	14.5	18.4	52	285	9.7	113	10.5	6.1	109	238			39	
7.3	193	16.0	12.0	48	258	7.3	234	19.2	21.0	51	306	7.3	138	11.7	0	43	193			42	

detoxicates it in the body. On the other hand, it has been shown that the parathyroid gland cures tetany by raising the blood calcium content. Some of the work on guanidine is vitiated by difficulties in distinguishing between it and creatinine.

Medes: Proc. Soc. Exptl. Biol. Med., 23:237 (1925).

Minot and Cutler: Proc. Soc. Exptl. Biol. Med., 26:607 (1929).



mann, and Wagner while working on parathyroid tetany found that guanidine compounds lowered the blood-sugar. Syntalin has been used as a substitute for insulin. It is poisonous in large doses.

Frank, Nothmann, and Wagner: Klin. Wochschr. 5:2100 (1926); 7:1996 (1928).

**Putrescine**, tetramethyl diamine,  $\text{H}_2\text{N}(\text{CH}_2)_4\text{NH}_2$ , is derived from arginine by putrefaction in the gut, with ornithine as an intermediate. Putrescine is found especially in the intestine and urine of patients suffering from cystinuria. It lowers blood pressure, and 0.1 mg. injected into a guinea-pig's vein is fatal. It is less toxic by mouth.

Dudley and Thorpe: Biochem. J. 19:845 (1925).

**Cadaverine**,  $\text{H}_2\text{N}(\text{CH}_2)_5\text{NH}_2$ , is formed by decarboxylation of lysine and was first found in cadavers. The lethal dose for a

guinea-pig is 0.1 mg. injected intravenously. It lowers blood pressure.

Orient: Biochem. Z. 132:352 (1922).

## EPHEDRINE SERIES

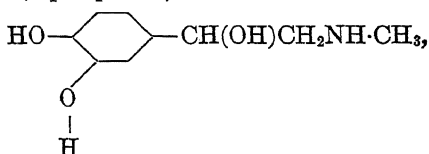
**Tyramine**, ergotamine,  $\text{HO}-\text{C}_6\text{H}_4-\text{CH}_2\text{CH}_2\text{NH}_2$ , is formed by the decarboxylation of tyrosine in the intestine. It occurs also in ergot, a fungus growing on the grain of cereals. Certain cephalopods on biting their prey inject tyramine from their saliva. Intravenous injection of 20–80 mg. raises the blood pressure.

Salant: Proc. Soc. Exptl. Biol. Med. 27:334 (1930).

**Hordenine**,  $\text{HO}-\text{C}_6\text{H}_4-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2$ , occurs in the embryo of barley. A dose of 500 mg. raises the blood pressure.

Abderhalden and Rossner: Z. physiol. Chem. 178:156 (1928).

***l*-Adrenaline**, epinephrine,



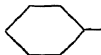
is not formed in the gut but in the medulla of the adrenal gland. In 1897 Abel isolated the benzoyl compound, and the free base was isolated by Aldrich and Takamine in 1901. An intravenous injection of 60 mg. in man was fatal. The effects of adrenaline are the same as those produced by stimulating the sympathetic nerves, and this has given rise to considerable difference of opinion between Cannon, who maintains that adrenaline is secreted in emotional states into the blood, and Stewart and Rogoff, who could not confirm all of Cannon's work. According to Cannon, adrenaline raises the hair of the frightened cat, causes first a dilatation of the pupil so as to allow a clearer view of his foe, then a contraction of the pupil sharpening the vision on the point of attack. It raises the blood pressure and increases the efficiency of the skeletal and heart muscles and increases blood-sugar; in other words, it is the fight-hormone.

Owing to its action in contracting the arterioles it is used for

bloodless surgery and to localize toxic substances, such as cocaine and snake poison, lessening their spread from the point of injection. Adrenaline solutions become colored by oxidation as is also the case with dihydroxyphenylalanine.


Cannon: *Physiol. Rev.* 9:399 (1929).

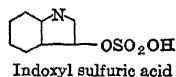
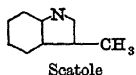
Stewart: *Physiol. Rev.* 4:163 (1924).

**l-Ephedrine**,   $\text{CHOH}\cdot\text{CH}(\text{CH}_3)\text{NHCH}_3$ , has a somewhat similar action. It is obtained from a Chinese plant, ma huang (*Ephedra vulgaris*). Its action is more prolonged than that of adrenaline probably owing to the fact that it is not so rapidly destroyed in the body. Adrenaline and ephedrine are given in asthma to dilate the bronchi. If too large doses of it are given, it interferes with urination.

Chen: *J. Pharmacol.*, 27:87 (1926).

### INDOLE SERIES

**Tryptamine**,   $\text{CH}_2\text{CH}_2\text{NH}_2$ , in certain doses raises blood pressure. It is formed in the intestine by decarboxylation of tryptophan. It is sometimes deaminized in the intestine with the formation of scatole and indole.



Indole is more toxic, the lethal dose being 3–5 mg. injected subcutaneously; but 1 g. by mouth has no effect on a dog. It is detoxicated in the body by conjugation with sulfuric acid to form:

**Indoxylsulfuric acid**, which is excreted in the urine and is detected by a color test. In medical literature it is called indican, but this is the name of a glucoside of indigo.

If to urine an equal volume of  $\text{H}_2\text{SO}_4$ , a drop of  $\text{CuSO}_4$ , and some  $\text{CCl}_4$  are added, when it is shaken, indigo blue will be formed and pass into the  $\text{CCl}_4$ . Sometimes a blue color is obtained and sometimes pink. The pink color in the  $\text{CCl}_4$  may be due to indigo red. Sometimes the color does not pass into the organic solvent, in which case it is due to still other substances, since indigo blue and indigo red are soluble in carbon tetrachloride.

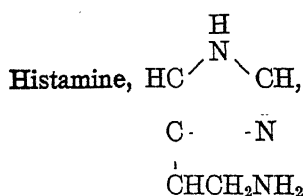
Nitrogenous bases and other protein decomposition products are called ptomaines, and formerly the indigo test was used as an index for ptomaine poisoning. Bacteria attack tryptophan, and the more tryptophan in the diet the more indoxyl  $\text{H}_2\text{SO}_4$  in the urine. Tryptophol and indole acetic and indole lactic acid also are produced by bacterial enzyme action on tryptophan.

Ewins and Laidlaw: *Biochem. J.* 7:18 (1913).

Fellers and Clough: *J. Bact.* 10:105 (1925).

Jackson: *J. Biol. Chem.* 73:523 (1927).

### IMIDAZOLE SERIES



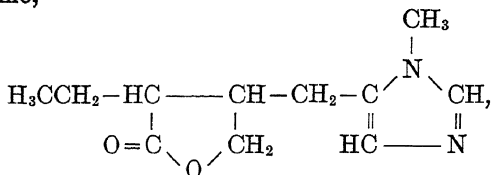
derived from histidine by decarboxylation, is a very powerful ptomaine physiologically. One milligram injected intravenously produces flushing of the face, lowering of the blood pressure, and marked gastric secretion, and in women stimulates the uterus. Larger doses produce symptoms of shock with increased permeability of the capillaries and decrease in blood volume. It is, therefore, a very toxic ptomaine.

A large amount of histamine is produced in the gut and from 5 to 20 mg. is excreted in the stools daily. Man tolerates 225 mg. by mouth. From 5 to 10 times the lethal injected dose can be drunk without causing death. It is destroyed by the gut wall; the liver also helps in its destruction, and the lethal dose in Eck-fistula dogs is smaller.

Histamine is formed by decarboxylase in tissue during the manipulation for the extraction of hormones, and either this or other toxic substances have caused death in early experiments on the injection of pancreatic and other hormone extracts. In such experiments it is wise to begin with small injections and note the blood pressure, before increasing the size of injections. If lowering of blood pressure occurs, histamine may be present.

Alvarez: *Physiol. Rev.* 4:352 (1924).

Wangensteen and Louchs: *Arch. Surgery* 16:1089 (1928).

**Pilocarpine,**

is a drug related to histamine.

Ets: Am. J. Physiol. 87:399 (1928).

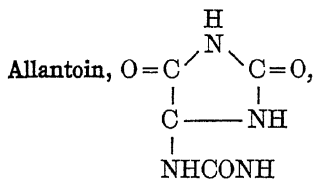
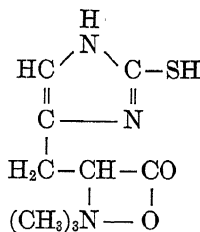
**Trimethylhistidine** occurs in mushrooms.

Reuter: Z. physiol. Chem. 78:167 (1912).

**Trimethylthiolhistidine**, ergothioneine, thio-  
neine, occurs in ergot and to the extent of  
10-25 mg. per 100 cc. blood. It was found in  
blood corpuscles by Eagles and by Benedict in  
1927.

Eagles and Cox: J. Biol. Chem. 80:249 (1928).

Hunter: Biochem. J. 22:4 (1928).

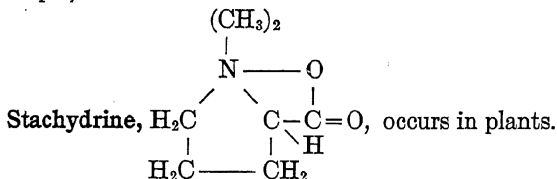


**Allantoin**,  $\text{O}=\text{C}-\text{N}-\text{C}=\text{O}$ ,  
is a base excreted by some ani-  
mals, which is supposed to be a  
connecting link between imid-  
azoles and purines. It is formed  
from uric acid by the action of  
*uricase* in the dog.

Morgan and Osburn: J. Biol. Chem. 66:573 (1925).

**PYRROL SERIES**

Pyrrolidines occur not only in proteins as proline but free, for  
example,

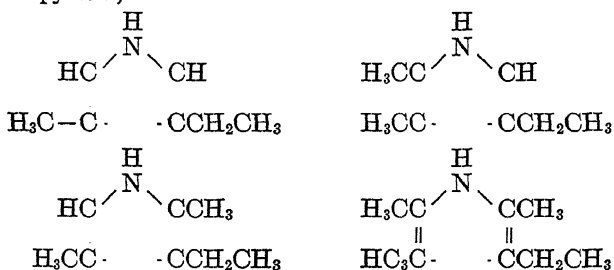


Yoshimura: Z. physiol. Chem. 88:334 (1913).

Pyrrols might be derived from proline or other constituents of  
the food. Four pyrrols are derived from hemoglobin, and the same

ones are derived from chlorophyll. There is a good deal of discrepancy as to their individual names, but they are known collectively as:

**Hemopyrrols,**



In the decomposition of hemoglobin of animals and chlorophyll of plants, other pyrrols may arise from these.

Willstätter and Asahina: *Ann.* 373:227 (1910); 385:188 (1911); *Ber.* 44:3707 (1911).

**Porphyrins** are decomposition products of both hemoglobin and chlorophyll. If they are injected into the blood they act as photosensitizers, and then ultra-violet light from the sun produces toxic effects.

**Phylloporphyrin**,  $\text{C}_{32}\text{H}_{36}\text{O}_4\text{N}_4$ , is found in buckwheat and photosensitizes animals fed on buckwheat.

**Hematoporphyrin**,  $\text{C}_{34}\text{H}_{38}\text{O}_6\text{N}_4$ , photosensitizes animals into which it is injected. These two porphyrins have similar absorption spectra.

Fischer and Zeile: *Ann.* 468:98 (1929).

Nencki and Zaleski: *Z. physiol. Chem.* 30:423 (1900):

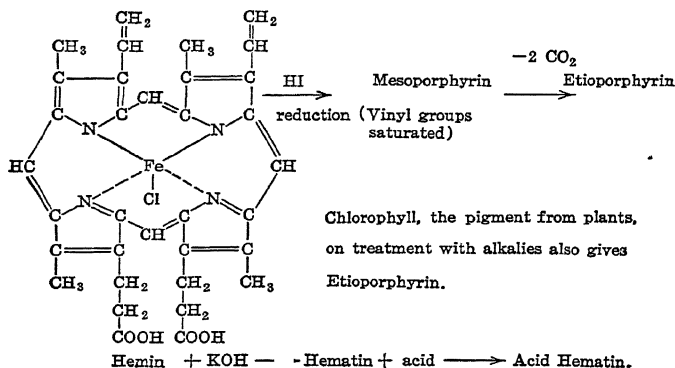
**Etioporphyrin**,  $\text{C}_{31}\text{H}_{36}\text{N}_4$ , has absorption bands centering at 6400, 6030, 5510, and 5910 Å in ether, and may be derived from chlorophyll or hemoglobin. Four isomeres have been prepared.

Fischer and collaborators: *Z. physiol. Chem.* 135:254 (1924); 142:141 (1925); *Ann.* 448:178 (1926).

**Etiophyllin**,  $\text{C}_{31}\text{H}_{34}\text{N}_4\text{Mg}$ , has absorption bands centering at 4980, 5434, 5791, and 6204 Å.

Willstätter and collaborators: *Ann.* 390:269 (1912); 380:177 (1911); 400:182 (1913).

**Hemin** is obtained from hemoglobin by using acetic acid and NaCl.



**Hematin**,  $\text{C}_{34}\text{H}_{32}\text{N}_4\text{O}_4\text{FeOH}$ , has been studied chiefly by means of the spectroscope without crystallization or analysis to show that the substance studied was  $\text{C}_{34}\text{H}_{32}\text{N}_4\text{O}_4\text{FeOH}$ .

Acid oxyhematin has absorption bands centering at about 6590, 5810–5610, 5335, and 2500 Å. When it is desired to purify the prosthetic group of hemoglobin, the most convenient method is to add chloride, in which case crystals (with absorption bands at 5570 and 5300 Å in 0.003% pyridine) having the composition  $\text{C}_{34}\text{H}_{32}\text{N}_4\text{FeCl}$  separate and are called hemin. The chlorine may be removed by hydrolysis, leaving the hematin.

The usual preparations of hematin for spectroscopy (as by dissolving hemoglobin in 0.1*N* HCl and allowing the solution to stand 30 minutes or more) contain protein. In fact, pure hematin is so poorly soluble that it would precipitate from such solutions. If pure hematin or hemin is to be used as a standard for colorimetry, gelatin or some other protein with which it combines must be added (Elvehjem).

Hematin will combine not only with protein but also with nitrogenous bases such as nicotine. The resulting compounds of reduced hematin are called **hemochromogens**. Some recent workers have avoided the name hematin and use the name **heme** for the prosthetic group of hemoglobin.

Keilin has shown that "heme compounds" are very widely distributed in both plant and animal cells under the name of **cytochrome** with three  $\alpha$  absorption bands (a 6046, b 5665, c 5592 Å) and also three  $\beta$  bands corresponding to three compounds a', b', and c' (as well as free heme).

Warburg showed that heme-nicotine is an oxidation catalyst and

that it is poisoned by carbon monoxide, and the compound with carbon monoxide, is dissociated by the action of light (in the same way that carbonyl hemoglobin is dissociated) of the same wavelengths as are *absorbed* by heme. Harrison showed that heme can oxidize cysteine to cystine.

In all the formulas of this series Willstätter gives 33 carbon atoms and Küster 34, which is usually accepted.

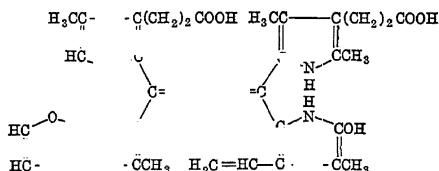
Anson and Mirksy: *Physiol. Rev.* 10:506 (1930).

Keilin: *Proc. Roy. Soc.* 98B:312 (1925).

Vernon: *Intracellular Enzymes* (1908).

Warburg and collaborators: *Biochem. Z.* 119:134 (1921); 202:202 (1928); 214:26 (1929).

### Bilirubin,



is a red bile pigment derived from hemoglobin and may be formed in any part of the body. It transmits light in the red-orange portion of the spectrum without showing absorption bands. The exact formula for bilirubin is not known, but it contains 4 pyrrol nuclei, and the above formula has been suggested.

Jaundice, a condition in which there is bilirubin in the blood, causes yellow pigmentation of the whites of the eyes. Van den Bergh claims that there are two kinds of jaundice, obstructive and hemolytic, which may be distinguished by blood chemistry.

Direct Van den Bergh test: Diazotized sulfanilic acid is added to blood serum. This gives a bright red color in *obstructive jaundice*.

In *hemolytic jaundice* red blood cells are destroyed. This form of jaundice cannot be detected by the Van den Bergh direct test since the bilirubin is masked in some way. In the *indirect Van den Bergh test*, blood is shaken with two volumes of alcohol, and then the direct test is applied to the alcohol solution. The alcohol may split off some protein (possibly lipoprotein) or may simply extract the bilirubin. There is not as much bilirubin present in hemolytic jaundice as in obstructive jaundice since there is a way for some of it to escape (bile duct).

If bilirubin is exposed to air, it is changed to **biliverdin** which is green. Not much is known about its composition, but it is claimed that it can be changed back to bilirubin. In some cases of jaundice the bilirubin changes to biliverdin. A "black eye" is due to biliverdin formed from extravasated blood.

In the intestine bilirubin is attacked by bacteria and changed to urobilinogen.

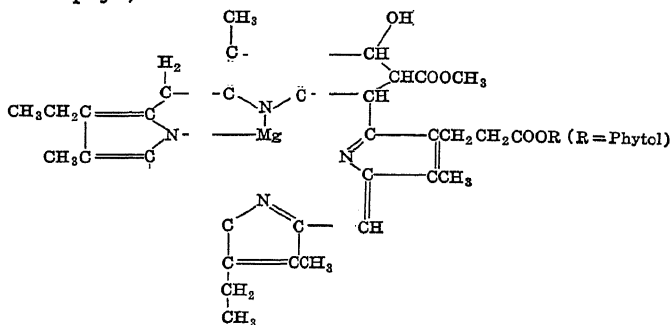
Küster: Ber. 35:1268 (1902).

Whipple: Physiol. Rev. 2:440 (1922).

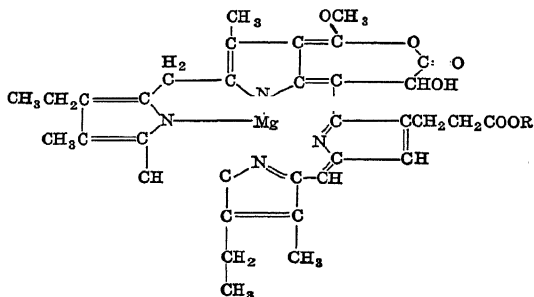
**Urobilinogen** is supposed to be formed in the intestine and to some extent to be absorbed by the blood and excreted in the urine. This may change over to some extent to **urobilin (stercobilin)**. This is partly excreted through the feces. In rare cases it is absorbed by the blood and excreted in the urine, hence the name urobilin.

Elman and McMaster: J. Exptl. Med. 42:619 (1925); 43:753 (1926).

### Chlorophyll,



Stoll's formula



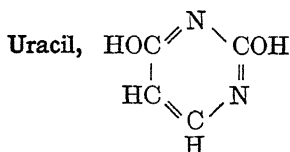
Conant's formula

Chlorophyll is a mixture of two substances; (a) with absorption band centering at 6850 and (b) 6685 Å.

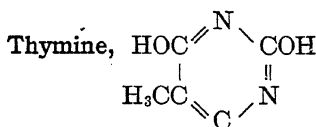
Willstätter and Stoll: Untersuchungen über Chlorophyll, Berlin (1913).

## PYRIMIDINE SERIES

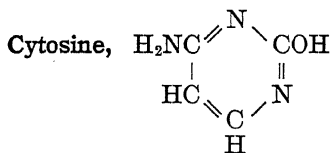
There are a number of pyrimidine compounds in the body. They occur mainly in nucleoproteins.



Wilson: J. Biol. Chem. 56:215 (1923).

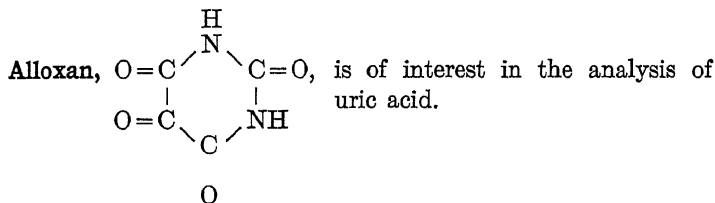


Baudisch and Bass: J. Am. Chem. Soc. 46:184 (1924).

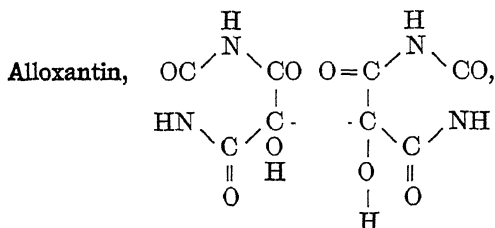


Calvery: J. Biol. Chem. 72:549 (1927).

These exhibit keto-enol isomerism, and the keto and enol forms are said to be in equilibrium with one another.

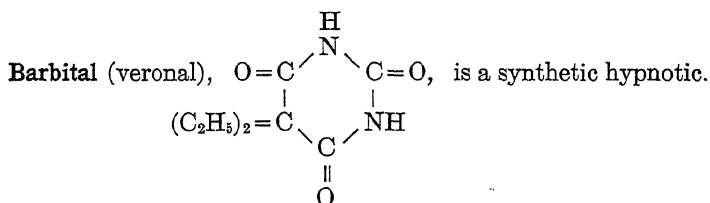


Biltz and Heyn: Ann. 413:60 (1916).

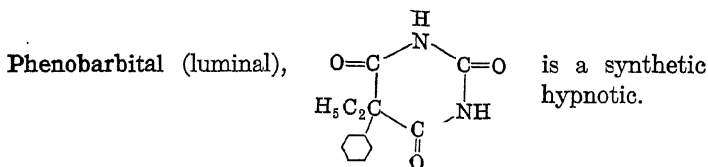


is a derivative of alloxan; it is also of interest in the analysis of uric acid.

Thunberg: *Skand. Arch. Physiol.* 33:217 (1916).

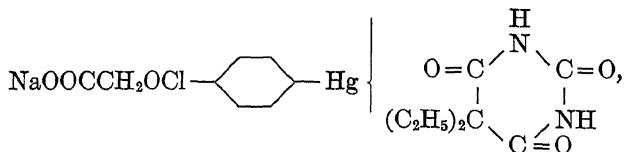


Eddy: *J. Pharmacol.* 33:43 (1928); 37:273 (1929).



Gruber and Roberts: *J. Pharmacol.* 27:327; 349 (1926).

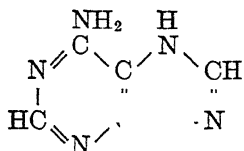
**Merbaphen** (novasurol),



is a diuretic. Its action is due to mercury liberation which prevents re-absorption of water by the convoluted tubules of the kidney (Sollman).

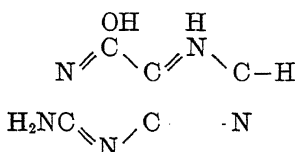
Rowntree: *Calif. and Western Med.* 3:12 (1929).

## PURINE SERIES

**Adenine,**

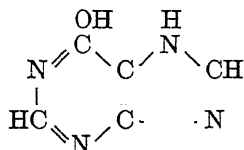
is widely distributed in organisms and has been mistaken for vitamin B.

Parnas: Klin. Wochschr. 7:1423 (1928).

**Guanine,**

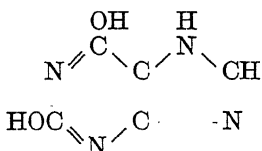
Pigs are subject to guanine gout, in which guanine crystallizes out in the joints and other tissues causing lameness and pain.

Voegtlin and Sherwin: J. Biol. Chem. 29:VI (1917).

**Hypoxanthine,**

may be derived from adenine but if oxygen is present uric acid is formed by the action of xanthine oxidase.

Back and Michlin: Ber. 60:82 (1927).

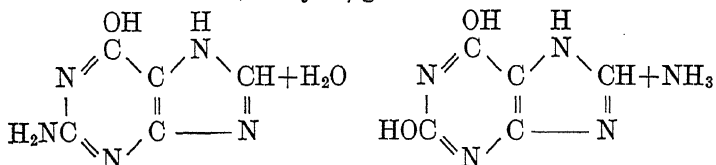
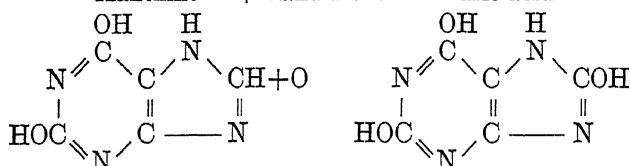
**Xanthine,**

is formed from guanine by the action of guanine oxidase.

Askew and Bruce: J. Agr. Sci. 19:573 (1929).

**Uric acid** is the end-product of nitrogen metabolism in birds and reptiles; urea is the end-product in mammals. Birds and reptiles drink very little water. Uric acid solution passes through kidney tubules where much of the water is re-absorbed and down into the cloaca, where the remaining water is re-absorbed. The uric acid crystallizes out since it has little affinity for water and is voided in semi-solid form (or solid pellets in "horned toad"). Only a few mammals, Dalmatian hound and primates, excrete uric acid, and probably only as a purine metabolic end-product, although Benedict's Dalmatian hound on a purine-free diet excreted uric acid.

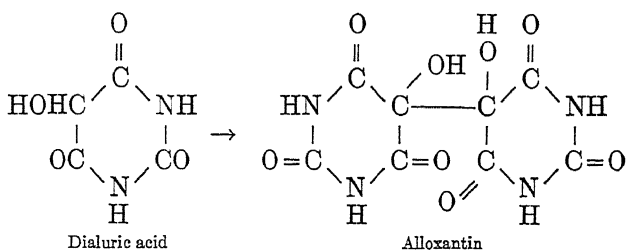
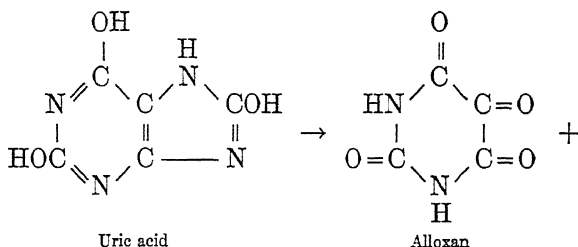
## PRODUCTION OF URIC ACID FROM PURINES

Guanine + enzyme, guanase  $\rightarrow$  xanthineXanthine + xanthinase  $\rightarrow$  uric acid

Normally there is 1-3 mg. uric acid per 100 cc. blood, but in nephritis it may go as high as 12 or more. Patients suffering from uremia usually have uricemia also. The amount of uric acid is dependent on the diet. If guanine occurs in the diet, uric acid occurs in the urine. On a purine-free diet, only 0.2-0.4 g. uric acid per day may appear in the urine.

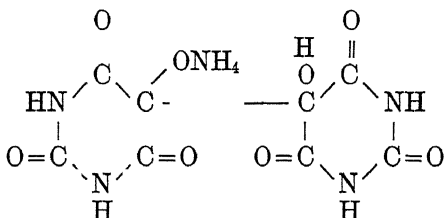
In man uric acid occurs in gout, characterized by uric acid crystals in joints and other tissues. Gout is thought by some to be due to too much uric acid in the body, causing it to precipitate out, since it is very insoluble in water (0.02% at 14°, 0.06% in hot water) whereas urea is very soluble in water. The amount found in urine will not dissolve in an equal volume of water, and the same is true of that in blood. Young and Musgrave found that piperidine solution would dissolve 20% uric acid at 90°. Sodium urate is more soluble than uric acid, a fact which is made use of in keeping it in solution. Lithium urate is much more soluble than sodium urate, and lithium used to be fed to patients with gout to dissolve the uric acid in the joints. If one takes a mixture of sodium and lithium salt, and then adds lithium urate, the sodium urate will precipitate. A man would die before he could substitute all the sodium in the blood by lithium.

## TEST FOR URIC ACID:



The alloxantin is treated with ammonia and ammonium purpurate is formed, called murexide, which gives a bright purple color to the test for uric acid.

Alloxantin + ammonia  $\rightarrow$  murexide

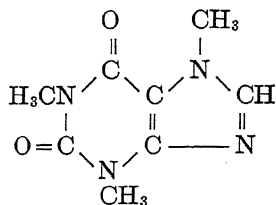


Levene showed that pyrocatechin interferes with the determination of uric acid.

Folin, Berglund, and Derick: J. Biol. Chem. 60:361 (1924).

Xanthine derivatives are of interest from the standpoint of some of the popular drinks.

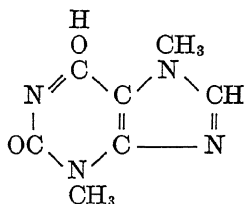
**Caffeine**, trimethyl xanthine,



occurs in tea, coffee and "coca-cola."

Preobraschensky: Arch. exptl. Path. Pharmacol. 132:330 (1928).

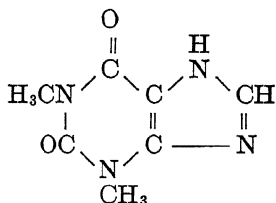
**Theobromine**, dimethyl xanthine,



occurs in chocolate.

Wallace and Pellini: J. Pharmacol. 29:397 (1926).

**Theophylline**,



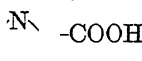
occurs in tea along with caffeine.

These xanthine derivatives may change one into another in the body, and may be changed partly to uric acid. Methyl xanthines are found in the urine after drinking much of them. They act as diuretics, theophylline being the strongest diuretic. Caffeine is a stimulant. One man drank 100 cups of coffee (possibly 10 g. caffeine) without ill effect whereas some persons are badly affected by about 1 g. The lethal dose is 13 g. The ordinary dose is about  $\frac{1}{3}$  g.

Hoffman: Arch. ges. Physiol. 220:124 (1928).

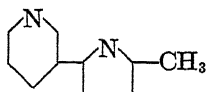

 PYRIDINE | SERIES

DeCaro: Arch. intern. Physiol. 29:163 (1927).

**Nicotinic acid**, , pyridine- $\beta$ -carboxylic acid, occurs

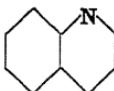
in many plants and has been mistaken for vitamin B.

Hunt and Renshaw: J. Pharmacol. 35:75 (1929).

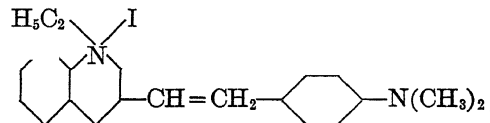
**Nicotine**, , 1-methyl-2- $\beta$ -pyridyl-pyrrolidine,

paralyzes certain nerve ganglia when a highly concentrated solution is applied to them locally.

Vickery: J. Biol. Chem. 84:233 (1929).

QUINOLINE  SERIES

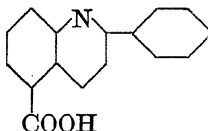
Darzens, Delaby, and Hiron: Bull. Soc. Chem. 47:227 (1930).

**Quinaldine red**, ,

is used as an indicator.

Kolthoff: Biochem. Z. 194:78 (1928).

McClendon: J. Biol. Chem. 59:437 (1924).

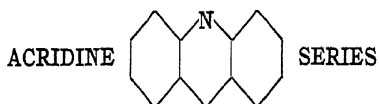
**Cinchophen**, atophan, , phenyl-quinoline car-

boxylic acid, is related to quinine. It has been given to patients with gout.

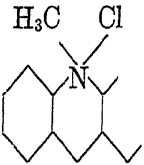
Stacy and Vanzant: Minnesota Med. 13:327 (1930).

**Quinine** is used to cure malaria. Its use was discovered by South American Indians.

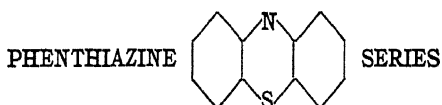
Kikuchi: J. Exptl. Med. 11:116 (1928).

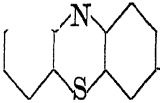


Nielson: J. Am. Med. Assoc. 91:1000 (1928); Acta Med. Scand. 70:12 (1929).

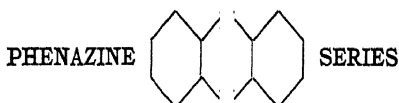
**Acriflavine**,  |, is a yellow dye that has antiseptic properties.

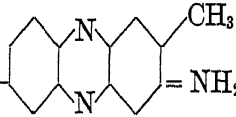
Jeck: J. Am. Med. Assoc. 93: (1929).



**Methylene blue**,  $(\text{CH}_3)_2\text{N}=\text{Cl}$    $-\text{N}(\text{CH}_3)_2$ , is an oxidation-reduction indicator.

Thunberg: Skan. Arch. Physiol. 35:163 (1917).



**Neutral red**,  $(\text{CH}_3)_2\text{N}-$    $=\text{NH}_2\text{HCl}$ , is an indicator.

Kolthoff: Rec. trav. chim. 43:144 (1924).

## PART IV

### *PHYSIOLOGICAL SUMMARY*

#### DIVISION 1

#### FOODS

**A. Animal Foods: MEAT.** Under polysaccharides there was a discussion of the differences between a carnivorous and herbivorous diet. The simplest diet in some ways is the carnivorous diet, and cannibalism should insure adequate nutrition. The body of closely related animals would be the nearest approach to the ideal diet. One difficulty with this diet is the eating of the skeleton. Furthermore, we have no enzymes with which to hydrolyze keratin, the protein of the outside covering of the skin, including hair and nails. With the exception of these and some other scleroproteins, meat leaves no residue on digestion. Small bones may be eaten if they are cooked in a pressure cooker, and the bones of canned fish are very soft. The muscles occupy the greatest bulk of the body, and muscle protein yields on hydrolysis the most economical proportions of the amino acids for our nutrition, but protein must not be considered the most important part of cold-storage beef, for instance. Stefansson, living a year under laboratory conditions on meat without any bones, obtained most of his calories from the fat. Furthermore, the fat contains vitamins A and D. It also contains unsaturated fatty acids, which are shown by Burr to be essential in nutrition. Other tissues, such as liver, brains, pancreas, and kidney, are richer in vitamins. Stefansson claims that a meat diet contains sufficient vitamin C but this has been found concentrated particularly in the adrenal. Well-bled carcasses are poor in sodium and if the bones are not eaten are deficient in calcium. A good many meats contain 10-20% protein. Thomas determined the biological value of foods in maintaining the nitrogen equilibrium in an adult and took milk protein as a standard, calling it 100. Beef was rated 104, fish 95, rice 88, potatoes 79, cheese 70, peas 56, wheat flour 40, corn meal 30.

MILK is the next most important food after meat, being deficient, however, in iron and copper, even though the cow is adequately

fed. It is said to be deficient in vitamin E for the reproduction of rats. Calves will not live more than nine months on their mothers' milk alone. Since its calorific value is about 310 Cal. per pound it might require 10-12 pints per day for an adult man. This would be more comfortable in summer than in winter.

The only food of general use that has about the right amount of mineral salts for the animal from which it is taken is milk, particularly milk of rapidly growing animals, in which case a close relation is shown between salts in the milk and salts in the animal. For instance, compare the milk of a bitch with the body of a pup. Ash them both, and 1000 grams of the ash will contain the following:

	Cow's milk	Dog's milk	Newborn pup	Human milk	Baby
K <sub>2</sub> O.....	269	150	111	314	78
Na <sub>2</sub> O.....	106	88	106	119	91
CaO.....	263	272	295	164	361
MgO.....	30	15	18	26	8
P <sub>2</sub> O <sub>5</sub> .....	330	342	394	135	389
Cl.....		169	84	200	77

Human milk does not show such close relationship, as the baby grows slower than the pup and most of the milk is used for energy and not for growth. Cow's milk is similar to dog's milk except for increase in potassium and magnesium.

Milk of individual cows is variable in composition (see table below), but state laws regulate the minimum values of mixed milk as sold as follows: Butterfat 2.5 to 3.5%, total solids 11.2 to 13%.

	MILK				
	Solids	Protein	Fat	Sugar	Ash
Cow.....	11.5-15.5	3-4	2.5-6	4.6-5	0.7-0.78
Goat.....	13.1	3.7-4.6	4.1	4.5	0.8
Human...	9-13.3	0.7-1.5	2-4	6-7.5	0.15-0.3

In modifying cow's milk to imitate human milk the protein is reduced by adding cream and water and the sugar is increased by adding lactose (or some other sugar such as glucose). The protein is not then of the same composition as in human milk (which contains a greater ratio of lactalbumin to casein than in cow's milk) unless it is further modified by adding soluble (not heat-coagulated) lactalbumin.

Storage of milk may lead to loss of vitamin C. Condensing milk in copper pans causes rapid destruction of vitamin C.

The phosphate in milk acts as a buffer, and cow's milk requires more acid to bring it to the isoelectric point of casein than human milk; therefore acid is sometimes added to cow's milk for infant feeding.

Cow's milk has more antirachitic potency than human milk, probably on account of its higher phosphate content.

Milk powder is a conveniently transportable form of milk. It may be added to bread and other foods and is of advantage in that the water content is low. By slow oxidation it acquires a tallowy taste.

CHEESE contains about 1% calcium and 0.7% phosphorus. It contains the casein and fat of the milk from which it was made. Some is made of skim milk.

EGGS must contain all the elements necessary for the tissues. They contain about 12% protein, 11% fat-like bodies, 0.5% carbohydrate, 1% ash, and about 75% water. The white contains water, protein and salts. The main nutritive constituents are in the yolk, which contains protein, lecithin, cholesterol, and an iron compound, "hematogen," and vitamins A, B, C, D, and G. The yellow color is carotin, but vitamin A may be present in a white yolk.

Lower animals yield on hydrolysis about the same constituents as the higher animals. Some of them may contain an indigestible carbohydrate, chitin, as in the shells of lobsters and crabs. If they contain hemocyanin instead of hemoglobin they are rich in copper. Some are rich in manganese. Those of marine origin are rich in iodine.

**B. Vegetable Foods.** Vegetable foods may be divided into two great groups, the cereal grains on one hand and other vegetables on the other.

CEREAL GRAINS are similar to meats in that on burning in the body or outside the body they yield an acid ash, due to the fact that the sulfur of the proteins is burned to sulfuric acid and the phosphoric acid of the conjugated proteins is uncombustible. Most of the cereal grain is endosperm (fig. 52), which consists mainly of starch with about 10% of a protein mixture of low biological value (gluten) and about 1% cellulose and about 2% pentosans. The ash contains enough phosphate but is deficient in

calcium, and iron is particularly absent. It is also deficient in sodium chloride and vitamins. Owing to these deficiencies whole grain bread has been advocated, since the bran and germ contain better proteins biologically, some fat, and vitamins B and E. There is not enough protein, however, to supplement the deficiencies in the amino acids in the endosperm protein (gluten), hence even whole grain bread has to be supplemented. The opponents of the whole grain bread idea point out the fact that there is much more nitrogen in the feces on a whole grain diet — in fact, the addition of the bran and germ to a diet of endosperm lessens the nitrogen retention rather than increases it. On the other hand, the large quantities of cellulose and pentosans in the bran may be undesirable.

Although from the nutritive standpoint the cereals are very closely related, there being minor differences such as the carotin content of yellow corn, the main differences in the cereals are in their milling and bread-making qualities. Bread can be made of wheat, rye, or barley and even oats, but not from Indian corn or rice. The mixture of prolamin and glutelin gives the gluten of the bread its bread-making qualities. Corn contains the prolamin and very little glutelin, and rice the glutenin and very little prolamin, but they cannot be mixed so as to form gluten that is good for bread making.

In threshing, the chaff is removed from wheat, rye, and corn, and when these are ground the germ and bran can be removed only by sifting (bolting). The germ of wheat and corn sifts out more easily than that of rye so that rye bread contains more of the germ. The chaff of rice, oats, and barley must be removed by milling. The primitive way was pounding it in a mortar, but the modern "paddy machine" consists of a hollow cone made of an abrasive material inside of which rotates a solid cone of the same abrasive, and the grains are ground between the two moving surfaces so as to remove the chaff. In this way white rice, pearl barley, and oats are obtained, although the oats are usually cooked and rolled before being sold. In this grinding process the germ is usually removed from rice. Some of it may remain in the oats and barley. It is for this reason that a rice diet is conducive to beriberi.

Patented breakfast foods are usually cereals sold at a higher price than bread but of the same nutritive value. In some cases bran is eaten. Hindhede, food controller of Denmark during the

war, claimed that a cereal and milk diet is the best adapted to human nutrition.

The digestibility of all vegetable foods is increased by milling, owing to the fact that otherwise poorly digestible and slowly fermentable carbohydrate prevents the digestive juices from reaching the starch and protein. Whole grain is made perishable on grinding because the fat-like substances in the germ become rancid. It is for that reason and not for principles of nutrition that the cereal grains have been degerminated very extensively. In the days of private and local mills degermination was not necessary. The American Indian first boiled the corn in lye or limewater to soften it and ground it by hand. The ground corn, "nixtamal," contained the germ. The Japanese rice was pounded in a mortar, "usu," worked by hand or sometimes by power. After the husk was broken off the pounding was not always continued to produce a pure white product and some of the germ was retained.

BEANS, PEAS, and NUTS are characterized by a very high protein content although their biological value is not always very high. There are two main classes of these seeds, those with a high starch content and those with a high fat content. Soy beans, peanuts, cottonseed and most nuts are high in fat. It was claimed by McCollum that cocoanut oil contained vitamin D, but in general vitamin D is in very low concentration in all natural vegetable foods, and vegetable oils are not good sources of it. The vitamin A content is quite variable. These seeds are known for vitamin B. All these seeds are very high in pentosans and are noted for the fermentation they produce. Not only are the carbohydrates fermented in the gut, but the entrance of the digestive enzymes into the cells is delayed and many of the proteins are fermented rather than digested, with the production of hydrogen sulfide from cystine.

ROOT VEGETABLES consist mainly of water and polysaccharides. In many of these the chief polysaccharide is starch. Mineral salts occur in moderate amounts.

LEAFY VEGETABLES are very high in mineral salts owing to the fact that the soil-water is sucked up into the plant and evaporated from the leaves, leaving the salts in the leaves. The potassium salts of deciduous leaves are sucked back into the plant before they fall but the calcium salts fall with them. Leafy vegetables are

higher in iodide, calcium, and iron than any other vegetable foods. They contain some protein but are mainly composed of water and polysaccharides. They may contain all of the vitamins but are rather poor in vitamin D, although they have received all the sunlight that is available. For some unknown reason the vitamin D content of dried leaves is increased by exposing them to the sun a few days whereas the living leaves have been exposed perhaps for months.

FRUITS are excellent sources for vitamin C since this vitamin in fruits is more resistant to cooking and drying and other modes of preparation than in other foods. During the World War, however, the Lister Institute showed that the vitamin C content of the bottled lime juice supplied the British vessels was almost nil. The fruits are high in sugars but they contain also considerable fermentable polysaccharides. They are also useful for their contained mineral salts. Some fruits, such as bananas and pears, may contain considerable starch.

## DIVISION 2

### DIGESTION

**Digestion in the Mouth.** The function of chewing is two-fold. In the first place, chewing increases the flow of saliva and this lubricates the food so that it may be easily swallowed. For animal foods this is all that is necessary. The boa constrictor swallows his food whole, and man could do likewise with animal foods were the esophagus large enough. In the second place, vegetable foods cannot be digested unless they are either milled or chewed, and in the ruminant the chewing process is long continued even after the food is first swallowed. Cooked starch is hydrolyzed very rapidly by the amylase (ptyalin) of the saliva. Amylase is absent from the saliva of carnivora unless they have been fed starch for a certain period of time. Perhaps one function of the amylase is the cleaning of the teeth, as starch left between them would otherwise ferment, forming acids which might etch the teeth. It has been recently shown that hard particles of food may be driven into crevices in or between the teeth and ferment, thereby giving rise to dental caries. Although the amylase may remove the starch if it is cooked, it is ineffective on the other polysaccharides or on starch inclosed in them, and hence fermentation may occur. Several liters of saliva are secreted a day. Saliva is about 99.4% water and contains the glycoprotein mucin as well as the enzyme amylase, and perhaps maltase. Its content of sulfocyanate may not be unusual, but this substance is more easily determined in the saliva than in other body fluids. When freshly secreted the saliva is slightly acid ( $pH$  about 6.6) but on opening the mouth it becomes alkaline owing to loss of carbon dioxide. On allowing saliva to stand some salts of the apatite series (calcium-phosphate) are precipitated along with cell detritus and mucin. This precipitate when formed on the teeth gradually hardens and produces the "tartar." The titrable alkali of the saliva varies from time to time and is increased by the presence of acids in the mouth. This probably protects the teeth from being etched by very acid food or drink. Saliva contains non-protein nitrogen constituents similar to those of blood. Hensch and

Aldrich found the salivary urea to average 80% of the blood urea, and Schmitz obtained a higher figure.

**Digestion in the Stomach.** The human stomach is an organ for storage and digestion of the food, and fermentation as a rule does not occur. In the ruminant the stomach is subdivided, part of it being an organ in which fermentation may go on. Before the discovery of hydrochloric acid in the stomach by Prout there was much debate as to whether the acid in the stomach was produced by fermentation or not. The influence of this debate is seen in modern times in the number of analyses for lactic acid that are made on gastric contents. The hydrochloric acid of the gastric juice will in time kill all fermentative organisms, and pepsin-hydrochloric acid will destroy many fermentative enzymes. Furthermore, the pH of the gastric content (1-2) is outside of the range of many fermentations. After the food is swallowed some time is required before the acidity of the gastric content reaches its optimum (fig. 23). During this time some fermentation, autolysis, and salivary digestion may take place, and in the infant's stomach milk is curdled during this period and the stomach empties before a high enough acidity is reached for peptic digestion. Although some pepsin is secreted it does not act until the milk reaches the pyloric region or duodenum. The adult stomach is, however, an organ for peptic digestion of proteins. The products of peptic digestion are called peptones but seem to be a mixture of polypeptides and proteoses. Some of the food usually remains in the adult stomach from 1 to 5 hours (in the infant's stomach 1 hour) and is partially liquefied in two ways, first in the addition of about 10 liters of fluid during the 24 hours (about 1.5 or more liters of swallowed saliva and about 8 or more liters of gastric juice); second, the peptic digestion of meat fibers, coagulated egg white, and other protein gels causes the food to be more liquid. After food enters the stomach the pylorus may close or become extremely narrowed. Contraction waves pass downward over the stomach wall and tend to drive the gastric juice and adjacent food toward the pylorus. Water drunk during a meal may pass along the lesser curvature out of the pylorus. The gastric contractions cause a spurting now and then of fluid contents into the duodenum. At the end of about 5 hours the pylorus may relax, allowing any solid particles that remain to pass out, but if they irritate the pylorus it may close again. It is possible, however, to pass a

6-g. metal weight on the end of a duodenal tube out of the pylorus at this time by lying on the right side with the hips elevated. Anything that causes the pylorus to remain spasmodically closed will cause "hyperacidity" due to the continued secretion of 0.1 N HCl without the eating of any more colloids that might bind the acid.

Boldyreff showed that regurgitation from the duodenum into the stomach may occur. If the stomachs of a number of people are pumped out before breakfast in some of them bile pigment will be found, which comes from the duodenum.

The digestion of meat in the stomach produces some substance which cures pernicious anemia. The stomach is probably a very useful organ, and no man has lived for many years without a stomach. Perhaps the main function of the stomach is to pass the food in small amounts at frequent intervals into the duodenum.

**Digestion in the Intestine.** The acid gastric content reaching the duodenum initiates the production of a hormone, secretin, which passes into the blood and causes the pancreas to secrete pancreatic juice containing protease (trypsin), lipase (steapsin), and amylase (amyllopsin). Bile also flows into the duodenum. Bile aids both in the emulsification of the fat and the absorption of the fatty acids liberated by the lipase. If the bile does not enter the small intestine, fat is digested but not absorbed. Besides pancreatic juice the intestinal juices from the glands of Lieberkuhn contain enzymes, particularly a mixture of polypeptidases known as erepsin, and invertase, which will hydrolyze any sucrose that has escaped the hydrochloric acid of the stomach. There is also in the intestine enterokinase, which is a proteolytic enzyme and which assists trypsin in proteolysis. Waldschmidt-Leitz supposes that it combines with trypsin to form trypsin-kinase. During digestion peristaltic waves or localized contractions pass over the wall of the intestine from the stomach to the cecum. The movements of the small intestine in man have not been very much studied owing to the fact that they are rapid, the intestine is over 20 feet long and thrown into many coils, which obscure one another when filled with bismuth subnitrate or barium sulfate to cast an X-ray shadow, and because of the danger of burning with too prolonged an X-ray exposure. The carnivora have a short intestine, and in the cat, so-called segmentating movements have been described in addition to the ordinary peristalsis, which tend

to churn up the food rather than drive it onward. These movements bring the food into contact with the villi, which are little finger-like processes of the intestinal wall whose function is to absorb the digested food.

The first part of the small intestine is the duodenum, or "twelve-finger gut," whose function is mainly to receive the bile and pancreatic juice and mix it with the food and the secretions of Brunner's glands. The second portion is the jejunum, or "empty gut," in which propulsion and absorption take place so rapidly that it is usually found empty when opened, particularly in the carnivora. The main part of the small intestine is the ileum, which may contain food residues and which is the main organ for the fermentation of polysaccharides. It used to be thought that the cecum of herbivora was the organ for fermentation, but it has recently been shown that herbivora ferment their food about as well after the cecum is cut out as before. In man part of the cecum is shrivelled up to form the appendix, but a man may ferment about as high a percentage of polysaccharides in his foods as does a cow. Herbivora are characterized by their ability to eat relatively large quantities of fermentable polysaccharides without the production of diarrhea. In the spring time their change in diet may produce diarrhea, which lessens their ability to utilize the food. Fermentation of food requires considerable time, and therefore the food has to remain in the ileum for a long period. Rapid propulsion defeats this object. If fermentable polysaccharides are fed to carnivora it requires only relatively small amounts to produce a diarrhea, and with the short intestine very little fermentation takes place. In man with a medium length of intestine a certain amount of fermentative digestion of food can take place, but many persons are sensitive to fermentable polysaccharides, and they produce either a diarrhea or spasm of the gut, which may be very painful. This is particularly noticeable in children who may experience colicky pain from eating green fruit. In the ripening of fruit some polysaccharide is changed to sugar which is easily absorbed.

Many devices have been tried for measuring the time required for food to pass through the intestine, but the general conclusion is that some portions may pass through more rapidly than others. Some of the food may stay several days in the small intestine, perhaps. Recently, with the use of X-ray studies, it has been

shown that persons in whom propulsion of food is rather slow feel better than those in whom it is rapid. Fermentation in the gut and its consequent fatty acids may be associated with rapid propulsion or spasm with the production of colicky pains (fig. 45).

Fermentation tends to prevent putrefaction in the gut. The products of putrefaction are called ptomaines and may be toxic when injected into the vein but apparently are non-toxic in the gut. Various amines, including cadaverine, indol, histamine, and choline, are produced. Histamine is quite toxic intravenously but probably of no significance in the gut. Acetyl choline increases the peristaltic movements, and thus it seems possible that putrefaction might increase propulsion.

What was formerly called ptomaine poisoning or autointoxication is now believed to be due to the thermolabile bacterial toxins of *Bacillus botulinus*, *Welchii* and *paratyphosus*, which may accumulate in stale food and cause sudden poisoning when it is eaten. Thus one need not wait for the incubation of paratyphoid fever but receives a sudden intoxication from large amounts of toxin in the food. If such food is heated to boiling all the way through immediately before it is eaten these symptoms will not occur.

Some persons are allergic to food, that is to say, they are sensitized to certain constituents, supposedly proteins. This has to be proved by specific tests.

**Digestion in the Large Intestine.** The colon including the cecum is an organ for the absorption of water from the food and for the excretion of certain substances and not a place for the digestion or absorption of nutriment. In diarrhea, however, considerable fermentation may take place in the colon leading to rapid propulsion and even spasm (fig. 42). The spastic colon may inhibit the elimination and be mistaken for slow propulsion. It is sometimes called "spastic constipation" and may be diagnosed by the X-ray after an enema of barium sulfate (clyster).

**Form of the Feces** is determined largely by the diet. In time of famine or on a meat diet without bones there is very little residue after digestion and absorption; defecation occurs very seldom and the feces may consist of black balls as in some herbivora. Greater bulk of the feces due to a herbivorous diet leads to the large cylindrical form (about  $1\frac{1}{4}$  inches in diameter). Fig. 41 shows a medium condition, that is with a moderate amount of residue. Since the colon is a region for the abstraction of water

from the food the longer it remains in the colon the drier it is, and then constipation, a condition shown in fig. 46, may occur. On the other hand, the spasm shown in fig. 42 may segment the food residue so that it is eliminated as in figs. 43 and 44, or in a long ribbon. The sigmoid and rectum are for the purpose of receiving the feces, and their distention leads to the act of defecation. If, on the other hand, this act is voluntarily inhibited, unpleasant symptoms arise which are known as symptoms of "constipation." Alvarez showed that these symptoms may be produced by packing the rectum and sigmoid with cotton gauze.

## DIVISION 3

### METABOLISM

The fats are absorbed as fatty acids in combination with bile salts; since the pH of the carnivorous intestine is always on the acid side of 7 (and fats play a larger rôle in the carnivorous diet than in the herbivorous diet) no soap can be formed and the fatty acids are insoluble in the free state; furthermore, if the bile does not enter the intestine the fatty acids are not absorbed but are excreted in the feces. Fatty acids are transmitted by the blood stream. By Bloor's method of fat analysis in blood, saponification is first performed so that the free and combined fatty acids are determined as a whole. Their concentration in normal blood is .45%. Certain sterols are also absorbed, for instance, cholesterol is; but the different phytosterols, as ergosterol, are not absorbed. The cholesterol content of blood is .2%. Fatty acids are metabolized in the liver and their iodine number increased. They may be here formed into phosphatides. The phosphatide content of the blood is .35%. If lower fatty acids up to caprylic are eaten they are built up into those of longer carbon chains in the body, but if the higher fatty acids are eaten they may influence the character of the fat in fat-depots to some extent. Thus, if a pig is fed on oil its pork will be "soft" because of the high iodine number of the oil raising the iodine number of the body fats. If it is fed on carbohydrate the fatty acids synthesized from carbohydrate have a low iodine number and a firm pork will be produced. Cholesterol, ergosterol, phosphatides, and cerebrosides are synthesized in the body.

Carbohydrates are absorbed chiefly as glucose, and only those are utilized that will change into glucose spontaneously in alkaline solution, that is to say galactose, mannose, and fructose. They pass through the portal vein to the liver. It is stated that fructose will form glycogen more readily than glucose. On fasting for 14 hours or more and eating 50 g. or more of glucose there is a sudden rise in blood-sugar from the normal level of about 0.1%, but on the glucose entering the duodenum there commences a formation of insulin in the pancreas, but this takes about  $1\frac{1}{2}$  hours to bring the blood-sugar to normal. According to Macallum the

mechanism is the formation of another hormone in the duodenum, which is absorbed in the blood and passes to the pancreas, causing the beginning of insulin synthesis. A second dose of glucose at the end of an hour and a half does not raise the blood-sugar of a normal person or raises it only slightly. In the diabetic, however, the second dose of glucose raises the blood-sugar to a higher level than the first dose. Furthermore, with the possible exception of what is called renal diabetes, the blood-sugar is always at a higher level than normal. This is due to the fact that there is an insufficiency of insulin. In most patients, however, the feeding of carbohydrate to the diabetic results in some utilization although it may be a relatively small amount, and it is not considered good practice to feed them only a carbohydrate-free diet or one very low in carbohydrate. Where does the sugar go that disappears from the blood on injection of insulin? It appears to be deposited as glycogen in the liver and then given up slowly as it is utilized by the body. Glycogen in the muscles does not raise the blood-sugar, and if the liver is cut out hypoglycemia results. If the muscles are exercised without sufficient oxygen to burn the lactic acid produced, lactic acid appears in the blood. A little may be excreted, but most of it passes to the liver and is formed into glycogen.

**Proteins** are digested to amino acids or peptides of low molecular weight before they are absorbed, leading to a rise in the amino nitrogen of the blood and the tissues. The normal value is .006% but it may rise to .01% after a meal. Part of these amino acids are deaminized and formed into urea in the liver, and part of the fatty acids resulting are formed into glucose. This process is accompanied by the evolution of heat, which is called the *specific dynamic action of protein*.

**Oxidation of Foodstuffs** is a very complicated process and requires a large number of enzymes, and it seems to be performed by a series of dehydrogenations and hydrations. The hydrogen acceptor seems to be glutathione, and the catalyst for the oxidation of the glutathione to be cytochrome, an iron compound related to hemin. According to Thunberg, all foodstuffs pass through the stage of succinic acid before oxidation is complete. Succinic acid is formed from two molecules of acetic acid. By a series of dehydrogenations and hydrations one molecule of succinic acid is broken down to one of acetic, and hence in this cycle one molecule of acetic acid is oxidized. If large amounts of fat are metabolized

butyric acid is not completely oxidized. It was shown by Knoop that fatty acids are oxidized by  $\beta$  oxidation, that is, two carbon atoms are cut off at a time. Thus oxidation of butyric acid gives rise to  $\beta$ -hydroxybutyric acid and  $\beta$ -ketobutyric acid. According to Snapper these substances are oxidized normally in the kidney, but the power of the kidney to do so is variable. In some cases of diabetes 100 g. of  $\beta$ -hydroxybutyric acid have been produced in the body in one day. Since  $\beta$ -ketobutyric acid is decarboxylated to form acetone, fatty acids of even number of carbon atoms are called *ketogenic*. Some fatty acids arising from the deamination of the amino acids are ketogenic. The others are changed into glucose. Glucose is said to be *antiketogenic* but perhaps by that is meant fat-sparing. It is not antiketogenic or fat-sparing in the diabetic. Fifty-eight per cent of protein is antiketogenic and 18% is  $-\text{NH}_2$ , which is eliminated as urea. The sulfur of cystine is burned to sulfuric acid. The elimination of sulfuric acid and phosphoric acid may cause the loss of base by the blood, which normally contains 0.03 *N* bicarbonate of alkali and alkaline earth metals (chiefly sodium bicarbonate), but this question will be taken up under Excretion. Inorganic salts form stimulating and depressing ions in the body fluids:  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{OH}^-$  being stimulating and  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , and  $\text{H}^+$  being depressing (except in case of respiratory and vasomotor centers, in which case  $\text{H}^+$  may be stimulating). The maintenance of a proper balance assures the physiological norm, and this maintenance is insured by the presence of vitamin D and parathormone as well as some other substances.

## DIVISION 4

### EXCRETION

Excretion occurs in the lungs, skin, and kidneys. In the lungs  $\text{CO}_2$  is excreted until the  $\text{CO}_2$  tension of the blood is equal to about 50 mm. of mercury. Since there are 125 sq. m. of surface in the lining of the lungs (alveolar spaces) the alveolar air is so close to equilibrium with the blood that for all ordinary analyses it may be assumed that equilibrium exists, and  $\text{CO}_2$  tension in the alveolar air is determined when that in the blood is desired. This  $\text{CO}_2$  comes from bicarbonate in the blood, and the base liberated would raise the pH if it were not for weak acids, chiefly in the form of proteins. Since most of the proteins are in the corpuscles, which contain about 40% hemoglobin, the corpuscle may be considered a huge polyvalent protein molecule, with this exception, however: it is covered with a membrane impermeable to potassium and sodium ions, and hence sodium liberated in the plasma by the escape of  $\text{CO}_2$  into the lungs cannot enter the corpuscles to combine with the hemoglobin. The process is a cycle and may best be begun in the tissues. Here  $\text{CO}_2$  enters the blood and part of it enters the corpuscles. Here it decomposes potassium hemoglobin, forming potassium bicarbonate. The  $\text{HCO}_3^-$  is then of such a concentration that it tends to diffuse into the blood plasma. In order to satisfy electrical equality  $\text{Cl}^-$  diffuses from the blood plasma into the corpuscles. From analyses then the  $\text{CO}_2$  is carried as bicarbonate in the plasma and the bicarbonate concentration in the plasma is increased without any more sodium or other cations entering it, that is to say, the main shift that shows in an analysis is the passage of chloride ions from the plasma to the corpuscles, decreasing the chloride content of the plasma and increasing that of the corpuscle. When the blood passes through the lungs the reverse takes place. Although the bicarbonate is in the plasma it cannot go out into the lungs directly but must diffuse as an anion into the corpuscle and be formed into the anhydride ( $\text{CO}_2$ ) and then pass into the alveolar air. When this takes place the potassium chloride of the corpuscles disappears owing to the passage of  $\text{Cl}^-$  out of the corpuscles and the union of  $\text{K}^+$

with protein (hemoglobin). The lungs also function for the oxygenation of hemoglobin of the blood, forming oxyhemoglobin which dissociates in the tissues giving up oxygen for oxidation.

The next most important excretory organ is the kidney, which is very complicated in nature and might be illustrated by mention of its comparative physiology. The kidney of some invertebrates is merely a tube allowing the fluid of the body-cavity to pass to the outside and in this way get rid of water from the body. Whether changes take place in the composition of this "nephridial" fluid has been very little studied. This nephridium might be compared to the contractile vacuole of the protozoa, eliminating water from the body. In the fish the chief excretory organ for things other than water is the gills, and the kidney is for the purpose of eliminating water. Some fish migrate between fresh and salt water and others are supposed to have migrated once or twice during their phylogeny. The gills come in direct contact with the water. In the shark family (elasmobranchs), the osmotic pressure of the blood is the same as the sea water, but more than half of it is made up by the osmotic pressure of urea which exists in concentration of 2-5%, and is eliminated by the gills when this concentration is exceeded. The urine of the shark does not have higher osmotic pressure than the sea water. If sharks are placed in fresh water they soon die, but it would be interesting to attempt to acclimatize them to water of extremely low salt content to see if the urea would disappear from the blood. If the urea is eliminated the blood would be of about the same osmotic pressure as that of the marine bony fish or little less than  $-1^{\circ}$  freezing-point. Some fishes migrate from the sea to fresh water, in which case there is a small drop in osmotic pressure of the blood, for instance from  $\Delta = 0.7^{\circ}$  in sea water to  $\Delta = 0.6^{\circ}$  in fresh water. In fresh water the bony fish absorbs water through the skin and gills and excretes a dilute urine. The kidney then is an organ to keep the body from being water-logged. The part of the kidney most active in the excretion of water is the glomerulus, in which blood passes through an ultra-filter, and this ultra-filtrate or glomerular fluid then passes through a convoluted tubule in which certain desirable substances in the ultra-filtrate are re-absorbed, leaving the water to pass out as urine. When this same fish migrates into sea water (as in the case of the eel) there is no longer any danger of the body becoming water-logged but on the contrary there is

danger of water being extracted from the body on account of the higher osmotic pressure of the sea water, and the fish has to work in order to obtain sufficient water. This is done by swallowing sea water, absorbing it, and excreting salt from the gills. The urine is then very scanty, as is also the case with reptiles and birds, which live under dry conditions. The function of the kidney in the elimination of water is well illustrated in the frog. If the mouth and cloaca are sealed and it is thrown into a pool it absorbs water until it bursts. This absorption of water occurs through the skin as is shown by tying the blood-vessels and lymphatics of the base of one leg so as to occlude them, in which case only that leg swells and bursts from the absorption of water. The blood in the other leg carries the absorbed water to the kidneys where it is excreted. All these lower animals excrete a urine which is either more dilute than or isotonic with the blood, but man may excrete a urine which is much more concentrated than the blood. This is due to possessing the loop of Henle, which absorbs water from the urine if water is needed in the body. How do the reptiles and birds dispense with the loop of Henle when they live under desert conditions? This they accomplish by precipitating out the osmotically active substances of the urine so as to lower its osmotic pressure. Urea is synthesized into uric acid which precipitates out in the urine, and so a semi-solid urine is excreted although it does not have a high osmotic pressure. In animals above the fish, gills are not present, in the adult at least, and most of the nitrogenous waste and salts must be excreted by the kidney. The kidney mechanism in man is pictured as follows: There is an ultra-filtration of the blood through the glomeruli. The quantity of the ultra-filtrate per day is not known but in one case of diabetes insipidus 50 liters of urine were excreted in one day, and it has been calculated that if certain dissolved substances are entirely excreted through the glomerulus it would require this amount or a little more, 67 liters of glomerular fluid. This ultra-filtrate passes through the convoluted tubules, in which the sugar is re-absorbed and where some of the salts that are needed in the body may be re-absorbed. It is supposed that some substances are excreted into the convoluted tubules, since the kidneys of certain marine fish, as the toad fish, have no glomeruli and yet excrete a little urea and considerable other nitrogenous waste. As the urine passes through the loop of Henle water is absorbed,

sometimes to such an extent that the osmotic pressure of the urine is greater than that of the blood. If the loop of Henle is poisoned by mercury, water is not re-absorbed and an enormous diuresis occurs. Still larger doses of mercury poison the glomeruli so that no urine is secreted. Richards and Beiter have analyzed glomerular fluid and watched the secretion of colored substances under the microscope, and Marshall has studied the comparative physiology of the kidney so that we feel more confident about this picture of urine secretion. Pituitrin causes an increase in the chloride excretion but a decrease in the water elimination, which probably means a greater glomerular filtrate but an increased action of the loop of Henle. The average daily excretion of urine is about 50 cc. per hour in a man and a little less in a woman, and since there may be 60 times as much urea in the urine as in the blood, Cushny has calculated that in the formation of urine 67 liters of blood plasma must filter through the glomeruli.

Urea secretion is greater in the day when the body temperature is high than at night when it is lower. Dehydration of the body may cause a fever, and hence the greater flow of urine may have something to do with the rise in body temperature. There is about the same amount of urinary pigment, urochrome, per day irrespective of the volume of urine, and if not enough water is drunk in order to cool the body and provide for the normal excretion of urine, the urine is scanty and highly colored, as is often the case in fever. If the patient were given sufficient water, however, a normal volume would appear. Particularly on a bread and meat diet the kidneys eliminate considerable excess acid over base from the blood, and if a sample of urine is titrated to the  $pH$  of blood, considerable alkali is necessary—in fact, Henderson and Palmer have found that the average value is 650 cc. of 0.1  $N$  alkali. This figure would be much higher if ammonia were not present. After the kidney has been excreting a highly acid urine for some time urea begins to be hydrolyzed in the kidney and ammonia excreted in the urine, which partially neutralizes it. This may be determined after titrating the urine to the  $pH$  of blood by adding formaldehyde, which will change the ammonia to the neutral hexamethylene-tetramine, and still more alkali must be added to bring it the second time to the  $pH$  of blood. Ordinarily, about as much is required the second time as was required to neutralize the titrable acidity. Before adding any alkali the  $pH$  of the urine

may be as low as 4.8. This pH is desirable in infections of the urinary tract, but it is possible that particular acids in it may be of significance in this regard. On a meat and bread diet the acidity of the urine is due to acid phosphates, but on a high-fat diet  $\beta$ -hydroxybutyric acid is excreted in larger amounts, (although it occurs in normal urine), and it is supposed that this acid is particularly bactericidal. Van Slyke and Palmer showed that normally a man excretes about 6 cc. of 0.1 *N* organic acid per kilo per 24 hours, which would account for about one-half of the titrable acidity. Some of this is  $\beta$ -hydroxybutyric acid, but there are also citric, glucuronic, oxalic, acetic, formic, butyric, benzoic, phenacetic, and phosphocarnic acid. After a meal HCl is excreted into the stomach and the urine may be alkaline for a short period. This is known as the *alkaline tide*. The nitrogenous constituents depend upon the diet. There may be 4–24 g. of total nitrogen, 3–20 urea nitrogen, 0.1–0.8 ammonia nitrogen, 0.6 creatinine nitrogen, 0.1–0.3 uric acid nitrogen, and 0.5–1 undetermined nitrogen in the normal urine. Uric acid depends on the purines of the diet and it is excreted during fasting and may be synthesized from histidine. Inorganic sulfate may be 0.6–2.8, ethereal sulfate 0.1–0.4; cystine sulfate 0.1–0.4, total sulfates 1–4, and sodium chloride 10–15 g. per 24 hours in normal urine. Crystals of leucine, tyrosine, and cystine may be present in normal urine. Cystine occurs in larger amounts in cystinuria. Urine of persons with melanotic tumors on exposure to air may develop melanosis (black pigment). The presence of albumin is considered a sign of nephrosis and nephritis. Postural albuminuria occurs in some individuals. The albuminuria occurs only in certain postures. It is supposed to be connected with the effect of hydrostatic pressure on the blood circulation. Bence-Jones protein is supposed to be a proteose which occurs in the urine in multiple myeloma. When urine is heated it precipitates but re-dissolves on raising the temperature further. Other proteoses and peptones occur in the urine in pneumonia, diphtheria, osteomalacia, and carcinoma. Creatine occurs in the urine under certain conditions, but owing to errors in many older analyses due to the interference of the acetone bodies, the results in cases of ketosis should be re-investigated.

During starvation the excretion of many substances falls. At the Boston Nutrition Laboratory a man fasted for a month. At the end of that time a 24-hour urine sample contained: total

nitrogen, 7 g.; amino nitrogen, 1.4;  $\beta$ -hydroxybutyric acid, 4 (0.2 before beginning the fast);  $\text{Na}_2\text{O}$ , 0.2;  $\text{K}_2\text{O}$ , 0.7;  $\text{Cl}$ , 0.12;  $\text{P}_2\text{O}_5$ , 1.3;  $\text{CaO}$ , 0.2; total sulfur, 0.5. During a prolonged fast the body lives on its own fat until this is exhausted, at which time there is a pre-mortal rise in urinary nitrogen due to the excessive burning of body protein, but this was not reached in this case. The titrable acidity of the urine was 220 cc. of 0.1 *N*.

**Excretion by the Gut.** During fasting about  $\frac{1}{2}$  g. of nitrogen is eliminated per day, which comes from the body probably first as mucin but later is partly formed into the bodies of bacteria. The large intestine is the place for the excretion of sterols, as observed by Schönheimer. Calcium phosphate is excreted by the lower bowel, particularly if the urine is alkaline, whereas if the urine is acid some of it passes out into the urine. The composition of sweat is probably very similar to that of glomerular fluid. Ordinarily about 0.3 g. of nitrogen is excreted in this way, and if we add 0.1 g. lost by the growth of hair, nails, and epidermis, and add this to that lost in the feces, there will be about 1.0 g. nitrogen lost per day in addition to that excreted by the urine.

**Internal Secretions.** The action of insulin and some other hormones has been mentioned in this summary. Perhaps the most active hormone is that of the thyroid, thyroxine. It not only affects markedly the metabolic rate, increasing it up to 100%, but it affects growth and differentiation. It is required, for instance, in order that a tadpole turn into a frog, although some strongly iodized proteins may have some effect after the thyroid is cut out. The parathyroid glands have to do with the blood-calcium level. The hypophysis secretes a number of hormones; the anterior hypophysis — growth hormone — controls the growth of the body, and there is an over-secretion of it in acromegaly. Besides this the hypophysis secretes a hormone stimulating the thyroid gland and a hormone stimulating the sex gland. The posterior lobe contains two hormones — pitocin, which causes the uterus to contract; and pitressin, which affects blood pressure and increases the chloride and diminishes the water in the urine. The adrenal medulla secretes adrenaline, which stimulates the organs controlled by the sympathetic nervous system and increases the blood-sugar. The adrenal cortex secretes a hormone whose absence causes Addison's disease. The pancreas secretes insulin. The thymus produces a hormone, and so may the pineal gland.

There are at least three female sex hormones — theelin, theelol, and also a hormone producing relaxation of the pelvic ligaments, and one causing pseudo-pregnancy when injected. There is at least one male sex hormone. Besides these the duodenum produces a hormone, secretin, which causes the pancreas to secrete pancreatic juice. According to Macallum there is a hormone which causes the pancreas to secrete insulin.

### ABBREVIATIONS OF JOURNALS

(The part italicized is the abbreviation)

- |  |   |
|--|---|
| <i>Acta Medica Scandinavica</i>  | <i>Chemistry &amp; Industry</i>   |
| <i>Agricultural Experiment Stations</i> (of the various states), <i>Bulletins</i> , <i>Circulars</i> and <i>Technical Papers</i> | <i>Chemical Reviews</i>   |
| <i>Agricultural Gazette of Canada</i>  | <i>Chemisch Weekblad</i>  |
| <i>Allgemeine Österreichische Chemiker und Techniker-Zeitung.</i>  | <i>Chemiker-Zeitung</i>   |
| <i>American Food Journal</i>   | <i>Comptes rendus hebdomadaires des séances de l'académie des sciences</i>  |
| <i>American Journal of Pharmacy</i>  | <i>Comptes rendus des séances de la société de biologie</i>   |
| <i>American Journal of Physiology</i>  | <i>Gazzetta chimica italiana</i>  |
| <i>American Journal of Science</i>   | <i>Helvetica Chimica Acta</i>   |
| <i>Annalen der Chemie</i> (Liebig's)   | <i>Industrial and Engineering Chemistry</i>   |
| <i>Annales de chimie</i>   | <i>Journal of Agricultural Research</i>   |
| <i>Archiv für die gesamte Physiologie des Menschen und der Tiere</i>   | <i>Journal of Agricultural Science</i>  |
| <i>Archives of Internal Medicine</i>   | <i>Journal of the American Association of Cereal Chemists</i> (Name changed Jan. 1924 to <i>Cereal Chemistry</i> ). |
| <i>Archives internationales de physiologie</i>   | <i>Journal of the American Chemical Society</i>   |
| <i>Archiv für pathologische Anatomie und Physiologie und für klinische Medizin</i> (Virchow's)                                   | <i>Journal of the American Medical Association</i>  |
| <i>Archives of Pediatrics</i>  | <i>Journal of the American Pharmaceutical Association</i>   |
| <i>Berichte der deutschen chemischen Gesellschaft</i>  | <i>J. of the Association of Official Agricultural Chemists</i>  |
| <i>Berichte über die gesamte Physiologie und experimentelle Pharmakologie</i>  | <i>J. of Bacteriology</i>   |
| <i>Biedermann's Zentralblatt</i>   | <i>J. of Biological Chemistry</i>   |
| <i>Biochemical Journal</i>   | <i>J. of the Chemical Society</i>   |
| <i>Biochemische Zeitschrift</i>  | <i>J. of Dairy Science</i>  |
| <i>British Association for the Advancement of Science, Reports</i>   | <i>J. of Experimental Medicine</i>  |
| <i>British Medical Journal</i>   | <i>J. of General Physiology</i>   |
| <i>Bulletin biologique de la France et de la Belgique</i>  | <i>J. of Metabolic Research</i>   |
| <i>Bulletin de la société chimique de France</i>   | <i>J. of Pharmacology and Experimental Therapeutics</i>   |
|  | <i>J. de pharmacie et de chimie</i>   |
|  | <i>J. of Physiology</i>   |

<i>J. für praktische Chemie</i>	<i>Seifensieder-Zeitung und Rundschau</i>
<i>J. of the Society of Chemical Industry</i>	über die Harz-Fett-, und Ölindustrie mit dem Beiblatt
<i>Klinische Wochenschrift</i>	<i>Sitzungsberichte Akademie der Wissenschaften in Wien</i>
<i>Lancet</i>	<i>Sitzungsberichte der preussischen Akademie der Wissenschaften</i>
<i>Monatshefte für Chemie und verwandte Teile anderer Wissenschaften</i>	<i>Skandinavisches Archiv für Physiologie</i>
<i>Philippine Journal of Science</i>	<i>Zeitschrift für allgemeine Physiologie</i>
<i>Physikalische Berichte</i>	<i>Z. für analytische Chemie</i>
<i>Physikalische Zeitschrift vereinigt mit dem Jahrbuch der Radioaktivität und Elektronik</i>	<i>Z. für angewandte Chemie</i>
<i>Physiological Reviews</i>	<i>Z. für anorganische und allgemeine Chemie</i>
<i>Quarterly Journal of Experimental Physiology</i>	<i>Z. für physiologische Chemie (Hoppe-Seyler's)</i>
<i>Recueil des travaux chimiques des Pays-Bas</i>	

## OTHER ABBREVIATIONS

Å = Ångström unit; a. = acid; alc. = alcohol; alk. = alkali; aq. = aqua, water; at. wt. = atomic weight; b.p. = boiling-point; Cal. = kilogram calorie; cm. = centimeter; cm.<sup>2</sup> = square centimeter; cc. = cubic centimeter; colorl. = colorless; cryst. = crystalline; d. = with decomposition; *D* = density (*D*<sub>4</sub><sup>25</sup> = at 25° with reference to water at 4°); eth. = ether; g. = gram; h. = hot; hex. = hexagonal; i. = insoluble; *K* = dissociation constant; *K*<sub>a</sub> = dissociation constant of acid; *K*<sub>b</sub> = dissociation constant of base; kg. = kilogram; l. = liter; mm. = millimeters mercury pressure; *M* = molecular weight; *M* = gram-molecule liter; m.p. = melting-point; m. = meter; m.<sup>2</sup> = square meter; m.<sup>3</sup> = cubic meter; mg. = milligram; min. = minute, mμ = millimicron; need. = needles; *N* = normal; pl. = plates; *P* = pressure; pH = log.  $\frac{1}{H^+}$ ; *R* = gas constant; s. = soluble; sl. = slightly; *T* = temperature centigrade; v. = very; *V* = volume; (α)<sub>D</sub> = specific rotation with light of D line of spectrum; α = degree of dissociation; γ = microgram (= 0.001 mg.); Δ = freezing-point lowering; μ = micron (micro-meter); μμ = micromicron; π = ratio of circumference to diameter of circle; ° = degrees Centigrade or angular degrees; % = per cent; ‰ = per thousand; [ ] = concentration or activity; < = less than; > = greater than.

The chemical symbols are given in the following table.

## INTERNATIONAL ATOMIC WEIGHTS, 1934

	Sym- bol	Atomic Num- ber	Atomic Weight		Sym- bol	Atomic Num- ber	Atomic Weight
Aluminum..	Al	13	26.97	Molybdenum	Mo	42	96.0
Antimony..	Sb	51	121.76	Neodymium..	Nd	60	144.27
Argon.....	A	18	39.944	Neon .....	Ne	10	20.183
Arsenic.....	As	33	74.91	Nickel.....	Ni	28	58.69
Barium.....	Ba	56	137.36	Nitrogen.....	N	7	14.008
Beryllium..	Be	4	9.02	Osmium.....	Os	76	191.5
Bismuth....	Bi	83	209.00	Oxygen.....	O	8	16.000
Boron.....	B	5	10.82	Palladium....	Pd	46	106.7
Bromine....	Br	35	79.916	Phosphorus...	P	15	31.02
Cadmium....	Cd	48	112.41	Platinum.....	Pt	78	195.23
Calcium....	Ca	20	40.08	Potassium....	K	19	39.096
Carbon.....	C	6	12.00	Praseodymium	Pr	59	140.92
Cerium.....	Ce	58	140.13	Radium.....	Ra		225.97
Cesium.....	Cs	55	132.91	Radon.....	Rn		222
Chlorine....	Cl	17	35.457	Rhenium.....	Re	75	186.31
Chromium...	Cr	24	52.01	Rhodium.....	Rh	45	102.91
Cobalt.....	Co	27	58.94	Rubidium.....	Rb	37	85.44
Columbium..	Cb	41	93.3	Ruthenium....	Ru	44	101.7
Copper.....	Cu	29	63.57	Samarium.....	Sm	62	150.43
Dysprosium..	Dy	66	162.46	Scandium.....	Sc	21	45.10
Erbium.....	Er	68	165.20	Selenium.....	Se	34	78.96
Europium...	Eu	63	152.0	Silicon.....	Si	14	28.06
Fluorine....	F	9	19.00	Silver.....	Ag	47	107.880
Gadolinium..	Gd	64	157.3	Sodium.....	Na	11	22.997
Gallium....	Ga	31	69.72	Strontium.....	Sr	38	87.63
Germanium..	Ge	32	72.60	Sulfur.....	S	16	32.06
Gold.....	Au	79	197.2	Tantalum....	Ta	73	181.4
Hafnium...	Hf	72	178.6	Tellurium.....	Te	52	127.61
Helium.....	He	2	4.002	Terbium.....	Tb	65	159.2
Holmium....	Ho	67	163.5	Thallium.....	Tl	81	204.39
Hydrogen...	H	1	1.0078	Thorium.....	Th	90	232.12
Indium.....	In	49	114.76	Thulium.....	Tm	69	169.4
Iodine.....	I	53	126.92	Tin.....	Sn	50	118.70
Iridium.....	Ir	77	193.1	Titanium.....	Ti	22	47.90
Iron.....	Fe	26	55.84	Tungsten.....	W	74	184.0
Krypton....	Kr	36	83.7	Uranium.....	U	92	238.14
Lanthanum..	La	57	138.92	Vanadium.....	V	23	50.95
Lead.....	Pb	82	207.22	Xenon.....	Xe	54	131.3
Lithium....	Li	3	6.940	Ytterbium....	Yb	70	173.04
Lutecium...	Lu	71	175.0	Yttrium.....	Y	39	88.92
Magnesium..	Mg	12	24.32	Zinc.....	Zn	30	65.38
Manganese..	Mn	25	54.93	Zirconium....	Zr	40	91.22
Mercury....	Hg	80	200.61				

# PART V

## LABORATORY WORK

### ACIDS, BASES, AND SALTS

Conc. (96%)  $\text{H}_2\text{SO}_4$  = 36 *N*  
 Glacial  $\text{CH}_3\text{COOH}$  = 17.5 *N*  
 Conc.  $\text{HNO}_3$  = 16 *N*  
 Conc.  $\text{HCl}$  = 12 *N*  
 Conc.  $\text{NH}_4\text{OH}$  = 15 *N* (sp. gr. 0.9)  
 Ammonium molybdate ( $\text{MoO}_3$ ) = 68 g. per liter (for  $\text{H}_3\text{PO}_4$ )  
 Magnesia mixture = 0.5 *N* (for  $\text{H}_3\text{PO}_4$ )  
 $\text{FeCl}_3$  = 2%  
 $\text{K}_2\text{CrO}_4$  = 5% (chloride indicator)  
 $\text{AgNO}_3$  (conc.) = 2 *N*  
 $\text{AgNO}_3$  (dilute) = 0.2 *N*  
 Sodium phosphate = 0.5 *N* (for *Mg*)  
 Stannous chloride = 1 *N* (reducing)

Indicators are usually 0.05% in 50% alcohol. Phenolphthalein is 1% in 95% alcohol.

### CLARK AND LUBS SERIES OF INDICATORS AT 15°

Indicator	Dissociation constant	$pK = \text{pH}$ at 50% dissociation
Thymol blue (acid range).....	$2.4 \times 10^{-2}$	1.62
Bromophenol blue.....	$1.0 \times 10^{-4}$	4.00
Methyl orange.....	$2.0 \times 10^{-4}$	3.70
Methyl red.....	$9.0 \times 10^{-5}$	4.05
Bromocresol blue (bromocresol green)..	$8.0 \times 10^{-6}$	5.10
Bromocresol purple.....	$8.5 \times 10^{-7}$	6.07
Bromothymol blue.....	$8.4 \times 10^{-8}$	7.08
Phenol red.....	$1.4 \times 10^{-8}$	7.85
Cresol red.....	$6.5 \times 10^{-9}$	8.17
Neutral red.....	$1.4 \times 10^{-7}$	6.85
Thymol blue.....	$1.1 \times 10^{-9}$	8.96

Since the reader has had some training in ordinary chemical analysis and probably very little or none in colorimetry, this section emphasizes analysis with a single instrument: the micro-colorimeter designed for the author by the Bausch and Lomb Optical Co. The cup

is so slender that 1 cc. fills it to a depth of 3 cm., and yet the author has used it for 10 years without breakage. When the unknown solution is set at 1 cm. depth (on the left) since the divisions on the vernier indicate 0.1 mm., the error of setting is considered less than 1%. These analyses may be made with other colorimeters, but in the determination of iodine the reduction of the size of the cup allows reduction of the size of the sample and saves much time in the burning of the sample.

When a color match is made, the reading of the depth of the standard solution (on the right) gives the concentration of the unknown directly. In order to reduce the error of reading to 1%, 10 readings are made and added, and the decimal point of the sum moved 1 place to the left. Thus all mathematical calculation is reduced to simple addition. Since the readings made by an experienced chemist do not ordinarily differ by more than 5%, any error in addition greater than 5% is eliminated by simple inspection.

**Use of the Duboscq Colorimeter** (Bausch and Lomb Micro-Colorimeter, fig. 53, or biological model with flare cups and compensation cups, 33-27-31-01, Hb standard, 33-23-21, 33-23-22, fig. 54). Remove the cover and raise the cups (the left one by loosening the knurled [milled] screw, raising the cup by hand, and tightening the screw; the right one by means of the rack and pinion). If the verniers do not read zero, adjust them, or record their readings so that corrections can be made. Place the colorimeter directly in front of the center of the window and move the mirror so that the two halves of the field in the eyepiece are white. If not of the same whiteness, move the colorimeter. If this does not make the two half-fields equal, clean the plungers and cups with lens paper. Do not set the bottoms of the cups on the table as they will become scratched but keep them in the cup-holders, which may be set on the table. Half fill the left cup with the unknown solution and move it up to touch the plunger. Half fill the right cup with the known solution (standard) and move up by means of the rack and pinion to touch the plunger (care must be used not to allow the cups to overflow). Look in the eyepiece; if a cup does not touch the plunger, that side of the field will be colored. Set the unknown solution at 1 cm. (or 2 cm.) and adjust known solution by means of rack and pinion until a color match is made. Repeat adjustment 10 times.

In making the calculation, it must be remembered that the *concentrations of the two solutions are in inverse ratio to their depths* when a color match is made with the eye, i.e., the concentration of the unknown is equal to the setting of the known divided by the setting of the unknown. Therefore it is more convenient to set the unknown at 1 cm. or some other whole number than to set the known at a

whole number, in which case the unknown may read an odd fraction when a color match is made. The majority of students make fewer mistakes when dividing by a small whole number than with long division, and if the unknown is set at 1 no division is made.

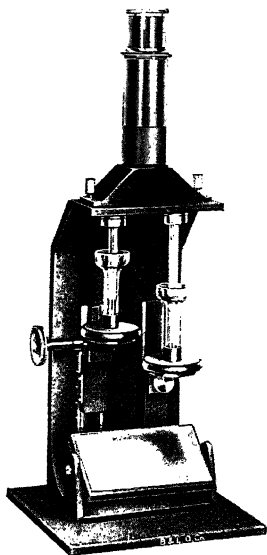


FIG. 53. B & L Micro colorimeter (cups for compensating color of unknown not shown but are shown in fig. 54).

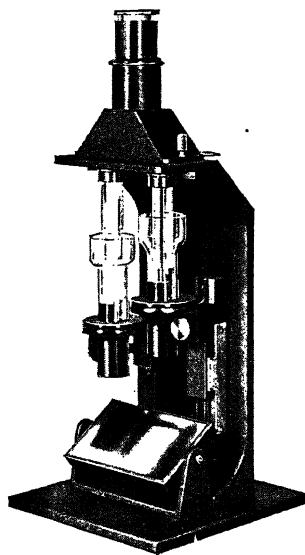


FIG. 54. B & L Biological colorimeter with cups for compensating color of unknown.

In case an indicator is put into a cloudy or colored unknown solution, set it at 2 cm. and place some of the unknown solution without indicator in the *compensation cup* under the known cup. These compensation cups are exactly 2 cm. deep when filled and the cover applied, and will fit either micro- or biological colorimeter. In this case the average of 10 readings is divided by 2. Compensation cups 1 cm. deep and of same diameter as flare cups of micro-colorimeter may be obtained.

**Analysis of Iodide in Acid Solution.** The solution is transferred to a 12-cc. separatory funnel with a mark at 10 cc. and diluted to the mark. One milligram  $\text{NaNO}_2$  and 1 cc. pure  $\text{CCl}_4$  are added and it is shaken 2 minutes. The  $\text{CCl}_4$  (containing not more than 85/95 of the iodine) is drawn off into a 1-cc. glass-stoppered centrifuge tube, centrifuged until clear, and placed in the left cup of the colorimeter

and set at 1 cm. A standard solution of 0.1 mg. of iodine per cc. is placed in the right cup, and the reading in centimeters gives the decimilligrams of iodine in the  $\text{CCl}_4$ . A second extraction is made with 1 cc. of  $\text{CCl}_4$  and the total iodine,  $x$ , in the sample analyzed is calculated from the following formula:

$$x = \frac{x_1^2}{x_1 - x_2}$$

in which  $x_1$  = iodine in first extraction and  $x_2$  in second extraction. Or divide  $x_1$  by  $x_2$  and find the percentage recovery in table.

$\frac{x_1}{x_2}$	Per cent of total in $x_1$	$\frac{x_1}{x_2}$	Per cent of total in $x_1$	$\frac{x_1}{x_2}$	Per cent of total in $x_1$
2.0	50.0	4.8	79.2	7.35	86.4
2.1	53.0	4.9	79.6	7.4	86.5
2.2	55.1	5.0	80.0	7.47	86.6
2.3	57.0	5.1	80.4	7.52	86.7
2.4	58.7	5.2	80.8	7.58	86.8
2.5	60.2	5.3	81.2	7.64	86.9
2.6	61.6	5.4	81.6	7.69	87.0
2.7	63.0	5.5	82.0	7.76	87.1
2.8	64.3	5.6	82.4	7.81	87.2
2.9	65.6	5.7	82.7	7.88	87.3
3.0	66.7	5.8	83.0	7.94	87.4
3.1	67.8	5.9	83.3	8.00	87.5
3.2	68.8	6.0	83.6	8.07	87.6
3.3	69.8	6.1	83.9	8.13	87.7
3.4	70.7	6.2	84.2	8.2	87.8
3.5	71.6	6.3	84.4	8.27	87.9
3.6	72.4	6.4	84.6	8.33	88.0
3.7	73.1	6.5	84.8	8.40	88.1
3.8	73.8	6.6	85.0	8.47	88.2
3.9	74.5	6.7	85.2	8.55	88.3
4.0	75.2	6.8	85.4	8.62	88.4
4.1	75.8	6.9	85.6	8.70	88.5
4.2	76.3	7.0	85.8	8.77	88.6
4.3	76.8	7.1	85.9	8.85	88.7
4.4	77.3	7.15	86.0	8.93	88.8
4.5	77.8	7.2	86.1	9.00	88.9
4.6	78.3	7.25	86.2	9.10	89.0
4.7	78.8	7.3	86.3		

**Ashing of Small Samples.** For iodine determinations, biological material is dried, powdered, weighed, moistened with a saturated alcoholic solution of lanthanum acetate, dried, and transferred to a glazed porcelain boat and ashed in a special combustion tube. The combustion tube (fig. 55) is made of a 200-cc. Pyrex balloon flask with hole in bottom admitting a silica tube 1 by 30 cm. containing an

electrically heated platinum spiral as combined catalyst and heater. The sample is burned in oxygen and the products of combustion sucked through a U-tube immersed in ice-water and containing about a gram of  $\text{NaHSO}_3$  in some water in the lowest portion of the U. The platinum coil is heated until luminous but only one end of the boat is introduced into it at first. As the sample burns, the boat is advanced until all of the sample is heated. If smoke results from combustion (often  $\text{NaCl}$  is volatilized forming smoke), this is filtered out through an Alundum capsule immersed in  $\text{NaHSO}_3$  solution. The  $\text{NaHSO}_3$  solution, ash, and washings of the tubes are evaporated to a small volume (if the ash is bulky, this is extracted repeatedly with alcohol in a ball mill, evaporated to dryness, and taken up in a little water), 1 mg. sodium azide added and neutralized with phosphoric acid to the yellowing point of brom phenol blue paper. It is boiled 1 minute to expel gases and analyzed as above.

**Ashing of Bulky Biological Material.** Although the above method is excellent for dried seaweed, thyroid, and other samples high in iodine content, a modification is necessary in order to handle a large bulk of the material, which could not be placed in the boat without a preliminary burning. Reith reports losses as high as 60% by open ashing. Therefore a stoking-apparatus must be constructed to prevent the whole sample from burning at once, as in such a case there will not be enough oxygen for complete combustion. If oils are used, an atomizer, supplied with oxygen instead of air, is desirable. Solid substances are dried and powdered, weighed, moistened with a saturated alcoholic solution of lanthanum acetate and dried, and placed in a Visking sausage casing which is fed into the combustion tube by means of a screw-feed device while inclosed in a nickel tube which conducts away the heat and prevents the combustion from creeping back on the sausage casing. A Pyrex liter balloon flask is substituted for the 200-cc. flask in the above apparatus. The screw-feed device is introduced into the Pyrex flask. The flask is set up horizontally, and the bottom opposite the screw-feed device is pierced by a hole into which is fitted the end of a silica tube about 30 cm. long and of 1-cm. bore. The further end of the silica tube is fitted into a Pyrex tube which bends in a U-shape downward into a beaker of ice-water, then down a second time through a 2-hole rubber stopper into an absorption flask. This absorption flask contains a large Alundum extraction thimble (RA 98) with a 1-hole stopper in the top connected with a large glass outlet tube which passes up through the 2-hole stopper and is enlarged into a 200-cc. bulb. This bulb is connected with a suction flask, which in turn is connected with a suction pump. About 3 m. of 24 gauge platinum wire is coiled in a spiral one end of which lies inside the Pyrex liter flask and the other end in

the silica tube. A 110-volt electric current is passed through the platinum wire and controlled by a rheostat so as to keep the platinum wire luminous but not melt it. If this temperature cannot be attained, part of the wire is cut off until a sufficiently high temperature is reached.

**Procedure:** The apparatus is set up in a horizontal position. Introduce the screw-feed device until its end reaches the middle of the Pyrex flask. Oxygen is admitted into one small nickel tube and gas into the other, and only enough gas is turned on to keep burning whereas the oxygen supply is as rapid as the suction apparatus will draw it through the tube. Wet asbestos is put around the screw-feed device as it passes through the neck of the balloon flask. The

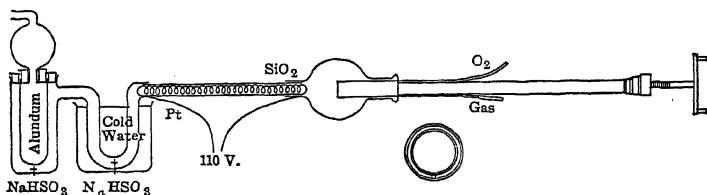


FIG. 55. Apparatus for burning dried tissue for iodine analysis. A detail of the opening of the screw-feed is shown below. The central circle is the space containing the dried tissue packed in a Visking sausage casing and around it is an annular opening from which oxygen issues to burn the tissue (the small circle below is an opening for a gas pilot flame). For burning tissue in a porcelain boat the screw-feed is removed and the platinum coils are extended through the pyrex flask for heating the boat (see *J. Biol. Chem.* 102:98, fig. 2).

silica tube is set up horizontally and one end inserted into the balloon flask, the other end into the Pyrex tube, the U of which is immersed in ice-water. The absorption flask is fitted on and the Alundum capsule filled with water containing 1 g. of sodium bisulfite, care being taken that the capsule touches the bottom of the absorption flask. The suction flask and suction pump are connected and a rapid stream of air is drawn through the apparatus. With the Visking casing containing the sample in the screw-feed device, the gas is lighted after pulling out the screw-feed device which is then reinserted and the screw is advanced until the flame ignites the end of the sausage casing. After the burning has gone backward as far as it will, the screw is advanced at such a rate that no tar is deposited in the balloon flask. A little white smoke will form and be caught by the Alundum capsule. This is largely alkali chlorides.

When the sample is all burned the ash may contain carbon, in which case it is then transferred to a boat and burned in the smaller

apparatus. If there is a little tar in the balloon flask, it is washed out with a little alcohol and evaporated in a combustion boat. The bisulfite solution and rinsings of the tube are evaporated in another combustion boat. These are all burned in the small apparatus. Sulfite solution is added to the ash and the rinsings of the tube of the second burning, and acidified with phosphoric acid in order to reduce any iodate with the sulfurous acid liberated. Then it is made alkaline with sodium sulfite or hydroxide, evaporated to complete dryness and extracted with alcohol in a ball mill and the alcohol evaporated. (Iso-propyl alcohol may be used instead of ethyl for the alcohol extraction.) If too large an amount of sodium chloride is extracted by the alcohol, it is necessary to evaporate the extract down and extract it again in a ball mill with a smaller amount of alcohol. The less water in the alcohol the less sodium chloride is extracted. Therefore, alcohol to which metallic sodium is added and distilled is preferable. The total amount of sodium chloride must not be more than a few milligrams. Since iodides are occluded in the salt, grinding in the ball mill must continue for over an hour. The alcohol is evaporated and the residue analyzed for iodide by the above method.

v. Fellenberg: *Biochem. Z.* 152:116 (1924).

Karns: *Ind. Eng. Chem. (Anal. Ed.)* 4:299, July 15 (1932).

McClendon: *Physiol. Rev.* 7:189 (1927); *J. Biol. Chem.* 102:91 (1933).

Reith: *Biochem. Z.* 216:249 (1929).

**Hydrogen-Ion Activity of Urine.** In the left cup of a colorimeter, place 0.05 cc. of 0.1% nitro-amino-guaiacol solution and 1 cc. of urine. In the right cup place 0.05 cc. of indicator and 1 cc. 0.1 *N* NaOH solution. Adjust the left cup to 20 mm. and place under the right cup a glass accessory cup containing urine 20 mm. deep. Determine the percentage dissociation (percentage color) in the left cup. If urine is highly acid, place over the eyepiece a glass dish with indicator in 0.01 *N* HCl. Read pH from the following:

% Dissociation	3.0	3.5	4.0	4.5	5.5	6.5	8.0	9.0	11.0	13.0			
pH	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5			
% Dissociation	15.0	17.0	19.0	22.0	25.0	29.0	33.0	37.0	42.0	46.0	50.0		
pH	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6		
54.0	58.0	62.0	66.0	70.0	74.0	77.0	80.0	83.0	85.0	87.0	89.0	91.0	92.0
6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0

McClendon: *Proc. Soc. Exptl. Biol. Med.* 21:348 (1924).

**Preparation of Nitro-Amino Guaiacol.** Place 90 cc. 10% NaOH in an Erlenmeyer flask and saturate with H<sub>2</sub>S. To 10 g. dinitroguaiacol add 20 cc. 10% NaOH and 200 cc. water. Heat to boiling, add the

$\text{Na}_2\text{S}$ , and boil gently for 10 minutes; add about 27 cc. concentrated  $\text{HCl}$  and cool. Filter off the precipitate and wash on filter with a little water, recrystallizing by dissolving in 1500 cc. hot water. Filter hot and cool. Filter off the crystals and dry.

Unpublished work of James B. Sumner of Cornell University, Ithaca, N. Y.

**Hydrogen-Ion Activity of Gastric Contents.** Filter and measure 1 cc. to which add 0.05 cc. of quinaldine red solution and transfer to left cup of the colorimeter. Add 0.05 cc. of quinaldine red to 1 cc. of water and place in right cup of colorimeter. Fill the accessory cup with gastric contents to compensate for color and turbidity, without allowing an air bubble in the cup. Set left cup at 20 mm. Match colors and read pH from the following table:

% Dissociation	2	3	4	5	6	7	8	9	10	14	20	24	29
pH	1	1.2	1.3	1.4	1.5	1.6	1.65	1.7	1.74	1.9	2.1	2.2	2.3
	34	39	44	50	56	61	66	71	76	80	91	99	
	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.7	4.7	

McClendon: J. Biol. Chem. 59:437 (1924).

**pH of Sea Water.** Pipet 0.02 cc. of saturated solution of *p*-nitrophenol (Schering-Kahlbaum) into each cup of colorimeter. Into left cup add 1 cc. sea water and into right cup 1 cc. 0.1 *N*  $\text{NaOH}$  solution. Compare in colorimeter and use accompanying table to find pH. (The intensity of color of the indicator solution changes on long stand-

Dissociation of <i>o</i> -Chrom-T	pH of solution with 1% salt (chiefly $\text{NaCl}$ ) (23°)	pH of solution with 3.5% salt (chiefly $\text{NaCl}$ ) (17°)	Dissociation of <i>p</i> -nitro- phenol (17°)	pH of solution with 3.5% salt (ocean water) 17°
0.02	5.00	5.70	0.02	6.9
0.03	5.20	5.90	0.03	7.1
0.04	5.30	6.00	0.04	7.2
0.05	5.40	6.10	0.05	7.3
0.06	5.50	6.20	0.06	7.4
0.07	5.60	6.30	0.07	7.5
0.08	5.65	6.35	0.08	7.55
0.09	5.70	6.40	0.09	7.6
0.10	5.74	6.45	0.10	7.65
0.14	5.90	6.60	0.14	7.8
0.1	6.1	6.7	0.2	7.9
0.3	6.3	7.0	0.3	8.2
0.4	6.5	7.2	0.4	8.4
0.5	6.7	7.4	0.5	8.6
0.6	6.9	7.6	0.6	8.8
0.7	7.1	7.8	0.7	9.0
0.8	7.3	8.1	0.8	9.2
0.9	7.7	8.5	0.9	9.7

ing leading to error.) *o*-Chrom-T may be used instead of *p*-nitrophenol but in this case  $\text{Na}_2\text{CO}_3$  must be substituted for  $\text{NaOH}$  in the standard.

**pH of Blood.** Draw blood under paraffin oil with enough 30% K-oxalate in needle to make 0.1% in blood drawn. Centrifuge in tube in which drawn. In each of two 10-cc. beakers place 0.1 cc. 0.1% *o*-Chrom-T. In the right beaker place 5 cc. 0.1 *N*  $\text{Na}_2\text{CO}_3$  and 2 drops 0.05 *N*  $\text{Ba}(\text{OH})_2$ . In the left beaker place 4.5 cc.  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ . Mix the left solution and pour into accessory cup. With needle point of special drawn-out pipet inserted to bottom of accessory cup inject 0.5 cc. plasma. Slide on cover without bubbles and rotate to mix and place on left side of colorimeter. Prepare a similar cup except for lack of indicator and place on right of colorimeter. Pour colored  $\text{Na}_2\text{CO}_3$  solution from right beaker into right plunger-cup of colorimeter and compare. Use first column in *o*-Chrom-T table.

McClendon: *Am. Naturalist* 64:289 (1930).

**$\text{NaHCO}_3$  (Alkali Reserve) of Blood Plasma.** Drawing blood: Do not use a tourniquet — if it is required to locate the vein, release before withdrawing the blood. Use a syringe and sharp sterilized needle. Transfer the blood to an oxalated centrifuge tube without removing the needle which, to prevent exposure to air, extends as near as possible to the bottom of the tube. Centrifuge within 2 minutes if possible. This procedure gives the same results as if the blood were drawn and centrifuged under oil.

Exposure to air while the cells and plasma are in contact will give low values owing to the Cl ion shifting from the cells, replacing the  $\text{HCO}_3$  ion, due to  $\text{CO}_2$  escaping from the blood.

The plasma may stand not more than 12 hours before the determination is made. On standing longer, the sodium of the glass may affect the result.

Place 1 cc. of oxalated plasma in each of two 200-cc. Pyrex flasks and add 20 cc. distilled water to each. Add 3 drops of phenol red to each flask. The color should be a reddish orange. If the color is not deep enough, add more indicator, but exactly the same quantities to each flask. Stopper one of the flasks, and to the other add 4 cc. of 0.01 *N*  $\text{HCl}$ . The color should now change to a yellow. Place this flask in the clamp on the end of a motor and rotate for 5 minutes while blowing in compressed air filtered through  $\text{NaOH}$  solution and then cotton. If during this period the color changes back to its original, add 1 cc. of  $\text{HCl}$  and continue the rotation for 3 minutes more. Titrate this flask back to the color of the stoppered flask with 0.01 *N*  $\text{CO}_2$ -free  $\text{NaOH}$ . It may be necessary to increase the volume by adding distilled water to the stoppered flask to get it to the exact

volume of the titration. After the reaction in the titration passes the isoelectric point of serum albumin and globulin, a precipitate will occur, but this should be more or less cleared up before the end of the titration is reached. The end-point is important because of buffers.

**Protein-Free Tungstic Acid Blood Filtrate.** Place 20 g. neutral anhydrous  $\text{Na}_2\text{SO}_4$ , 10 cc. 10% sodium tungstate solution, and 10 cc.  $\frac{2}{3} N$   $\text{H}_2\text{SO}_4$  in a 250-cc. volumetric flask, add water to dissolve, and dilute to mark. Transfer 2 cc. to a 2.5-cc. centrifuge tube, add 0.02 cc. blood (rinsing the pipet with the solution) and keep stirred 15 minutes for diffusible constituents to escape from the corpuscles. Centrifuge and aliquot. Larger quantities may be handled in the same way.

Folin and Svedberg: J. Biol. Chem. 88:85 (1930).

Folin: J. Biol. Chem. 51:419 (1922).

**Sodium.** Place 0.2 cc. blood in 3 cc. water in a 15-cc. centrifuge tube and stir in 0.8 cc. 20% trichloroacetic acid. Centrifuge and place 2 cc. supernatant fluid in a second centrifuge tube. Place 2 cc. standard sodium solution in a third tube. To each of the two tubes stir in 6 cc. of freshly filtered uranyl zinc acetate reagent and 0.3 cc. alcohol. After the bulk of the precipitate has settled stir in another 0.3 cc. alcohol without disturbing the precipitate. This is repeated 7 times. Centrifuge and decant. Wash precipitate once by mixing with 5 cc. of freshly filtered wash solution. Centrifuge and drain the tube, wiping off the mouth with a moist cloth. Add a drop of glacial acetic acid and rinse the walls with 10 cc. distilled water. The precipitate is dissolved and washed with 3, 10 cc. portions of water into a 50-cc. volumetric flask and diluted to volume. A 10-cc. aliquot is removed and placed in a large test tube with 10 cc. of water. Add 0.5 cc. of 20% potassium ferrocyanide solution. The unknown and standard are compared in a colorimeter.

**Uranyl Zinc Acetate. Solution A:** Mix 80 g. of sodium free uranyl acetate (Bakers Analyzed), 46 cc. of 30% (by volume) acetic acid, and water to 520 g. **Solution B.** Mix 220 g. of zinc acetate, 23 cc. of 30% acetic acid and water to make 520 g. The above are heated on a steam bath with occasional stirring until dissolved. Mix A and B while hot and filter after 24 hours.

**Wash Solution:** 1 g. of sodium uranyl zinc acetate is just moistened with distilled water and a liter of glacial acetic acid added with mixing.

**Standard Sodium Solution:** Dissolve 5 g. pure  $\text{NaCl}$  in 500 cc. water. 1 cc. = 3.9 mg. of Na.

J. B. C. 96:659 (1932).

**Sodium in 0.1 cc. Serum or Blood.** One cubic centimeter of 1% potassium pyroantimonate in 0.1% KOH is placed in a Pyrex centrifuge tube. Add 0.1 cc. of serum (or the ash of 0.1 cc. of blood which has been dissolved in 0.5 cc. of 0.1 *N* HCl and alkalized with several drops of sodium-free 10% KOH). To this add 1/5 of the entire volume of absolute alcohol, drop by drop, and stir constantly with a rubber-tipped rod (policeman). Allow this to stand for 45 minutes and centrifuge at 2000 r.p.m. Wash pipet with 2 cc. 50% alcohol several times and then dissolve in 0.5 cc. HCl ( $D = 1.15$ ). This is now placed in a 25-cc. graduated test tube. In a similar tube 3 cc. of the standard sodium pyroantimonate solution (2 cc. for the blood) is added. Add to both tubes 10 cc. water, 3 cc. of 10% gelatin, and 2.5 cc. 10% sodium sulfide solution. Mix and fill to mark with distilled water. Compare in colorimeter.

Standard sodium pyroantimonate solution: 0.1108 g. sodium pyroantimonate is dissolved in 125 cc. HCl ( $D = 1.15$ ) and made up exactly to 100 cc. with distilled  $H_2O$ ; 1 cc. = 0.1 mg. Na. (Reagent is stable for 1 month at room temperature.) The sodium pyroantimonate is prepared by adding 50 cc. 2.5% NaCl solution to 500 cc. potassium pyroantimonate solution, and add to this  $\frac{1}{2}$  of volume of 95% alcohol — a precipitate results. This is filtered and washed several times with 50% alcohol and dried in an oven at  $110^\circ$  to constant weight.

Yoshimatsu: Tohoku J. Exptl. Med. 8:496 (1927).

**Potassium** is determined by precipitation with sodium cobaltinitrite from acetic acid solution of an ash or directly from blood.

Measure 1 cc. serum into a 15-cc. centrifuge tube. Add slowly drop by drop 2 cc. sodium cobaltinitrite reagent of pH 5.7 and mix thoroughly. After 45 minutes add 2 cc. water and mix thoroughly, centrifuge at speed of 1300–1400 r.p.m. for  $\frac{1}{2}$  hour. Syphon off all the supernatant fluid except 0.3 cc. by using a syphon tube, the lower end of which is curved so that the opening is directed upward. Do not disturb precipitate. Allow 5 cc. water to run down the side of the tube, agitating by a circular motion to aid in mixing. Centrifuge for 5 minutes. Repeat until the precipitate is washed 4 times in all. The supernatant fluid of last washing should be clear. Syphon it off; add an excess of 0.02 *N*  $KMnO_4$  (2 cc. is sufficient for normal serum). Add 1 cc. 4 *N*  $H_2SO_4$ . Thoroughly mix with a glass rod. Heat in a boiling water bath until no further change in color can be observed. Compare in colorimeter with 0.02 *N* potassium permanganate. The total number of cubic centimeters of 0.01 *N* potassium permanganate required to oxidize the potassium cobaltinitrite times 7.1 = mg. potassium per 100 cc. serum.

Kramer and Tisdall: J. Biol. Chem. 46:339 (1921); 41:263 (1920).

**Magnesium in Tungstic Acid Filtrate of 1 cc. Oxalated Blood** (Calcium-free). (Calcium oxalate precipitates best between pH 4 and 5.6.) (Yoshimatsu has modified the method so that removal of calcium is not necessary.) Precipitate with *o*-hydroxyquinoline, wash, dissolve in HCl, determine *o*-hydroxyquinoline with Folin's phenol reagent —  $\text{Na}_2\text{CO}_3$ . Make tungstic acid blood filtrate as for calcium analysis (see below). A standard solution of magnesium *o*-hydroxyquinoline dissolved in HCl is run through the analysis along with the precipitate from blood filtrate and compared in a colorimeter.

Yoshimatsu: Tohoku J. Exptl. Med. 14:29 (1929-30).

Greenberg and Mackey: J. Biol. Chem. 96:419 (1932).

**Magnesium in Bone.** From 250 to 500 mg. fat-free bone powder is transferred to a beaker, moistened, and 5 cc. 5 *N* HCl is added. This is filtered into a 100-cc. volumetric flask. The precipitate and filter are then washed with water and flask filled to the mark; 20 cc. of this bone-solution is transferred to a 50-cc. centrifuge tube and 10 drops of dibromcresolpurple solution are added and then 20 cc. of a saturated solution of ammonium oxalate. Concentrated ammonium hydroxide is added drop by drop until pH 3-6 is reached. The sample is then allowed to stand overnight. Next day the precipitate is packed down by centrifuging. The filtrate is decanted, evaporated to about 10 cc., and transferred to a 50-cc. centrifuge tube which tapers to a point. One cubic centimeter ammonium citrate reagent and 3 cc. 10% ammonium phosphate are added, followed by about 8 cc. concentrated ammonium hydroxide. If separation of crystalline ammonium magnesium phosphate does not begin at once, the sides of the tube are scratched with a glass rod, and the tube is allowed to stand overnight. The next day a drop of alcohol is added to throw down crystals from the surface and it is centrifuged at high speed. Ammonium hydroxide diluted 4 times is added up to 40 cc. and the tube again centrifuged; all but 0.5 cc. of fluid is separated as before and the entire washing repeated. After the second washing as much of the supernatant fluid as possible is removed without disturbing the precipitate. Three cubic centimeters 20% trichloroacetic acid and 10 cc. water are added and after solution of the precipitate is complete, the sample is transferred to a 100-cc. volumetric flask and there are added: 10 cc. molybdic acid reagent, 5 cc. 20% sodium sulfite, 5 cc. 0.2% hydroquinone solution, and water to 100 cc. The standards containing 0.5 and 1 mg. phosphorus are prepared in the same manner for comparison with the unknown in colorimeter.

Kramer and Howland: J. Biol. Chem. 68:711 (1926).

**Calcium in Blood.** Pipet 0.1 cc. blood into a 5-cc. conical centrifuge tube containing 0.7 cc. distilled water. Mix to lake and add 0.1

cc. 10% sodium tungstate solution and mix. Add slowly 0.1 cc.  $\frac{2}{3}N$   $H_2SO_4$  and mix with a glass rod. After standing for 15 minutes, centrifuge and aliquot. To the aliquot add 0.3 cc. 33% seignette salt (KNa tartrate) solution and 0.5 cc.  $N$  NaOH solution and mix well, then add 0.3 cc. 5% alcoholic *o*-oxyquinoline solution drop by drop and mix. Wait 10 minutes, then stir vigorously with a fine glass rod for about 2 minutes until the turbidity reaches its maximum. Then dip in a boiling water bath for 2 minutes. Cool to room temperature and wait 15 minutes, stirring occasionally to complete the precipitation of calcium, centrifuge until precipitate is packed hard, decant the supernatant fluid, and wash 4 times with 1 cc. alkaline seignette salt solution. Decant, then add 1 cc. ammoniacal ammonium chloride solution and stir. Then place the centrifuge tube in a water bath of  $80^\circ$  and gradually raise the temperature to the boiling-point. Wait 1 minute, then add 1 drop ammonia water and mix. After 3 minutes add again 1 drop of ammonia water ( $D = 0.96$ ). Wait another minute and then add 0.5 cc.  $0.01N$  HCl. In another 10-cc. volumetric tube pipet 2 cc. (0.01 mg. Ca) of standard solution, one drop of ammonia water, and a few cubic centimeters distilled water. Add to each 1.2 cc. 20% sodium carbonate solution and mix. Then add 1 cc. phenol reagent to each. Place the flasks in a boiling water bath for 5 minutes; cool to room temperature. Make up to mark and mix. Compare color in colorimeter.

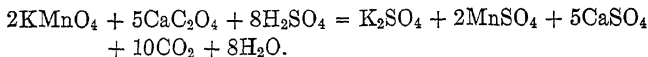
*Standard Solution.* Place about 5 g. seignette salt in 100 cc. 1% pure  $CaCl_2$  solution, then add 25 cc.  $N$  NaOH and mix. Add a few cubic centimeters 5% alcoholic *o*-oxyquinoline solution and mix well. Wait  $\frac{1}{2}$  hour, then collect the calcium oxyquinoline precipitate on a Büchner funnel and wash several times with 5% alkaline seignette salt solution. Dry at  $120^\circ$ . Take 0.41 g. of this calcium oxyquinoline, dissolve it in 50 cc.  $N$  HCl, and dilute to 500 cc. Of this stock solution, to make the standard, take 25 cc. and dilute to 500 cc. Two cubic centimeters of this solution equals 0.01 mg. Ca.

*5% Alkaline Seignette Salt Solution.* Dissolve 5 g. seignette salt in 100 cc. distilled water and add 20 cc.  $N$  NaOH.

*Phenol Reagent.* Mix 750 cc. distilled water, 100 g. sodium tungstate, 20 g. phosphomolybdic acid, 50 cc. 85% phosphoric acid; boil gently for 2 hours. Dilute to 1 liter.

Yoshimatsu: Tohoku J. Exptl. Med. 15:355 (1930).

**Blood Serum Calcium.** Calcium is precipitated as calcium oxalate and the oxalate is titrated against potassium permanganate. Calcium precipitates as the oxalate at a  $pH$  of 4. Magnesium precipitates at a  $pH$  greater than 4. It is necessary to have the  $pH$  at this point for complete separation of calcium and magnesium.

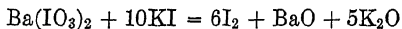


The blood should be drawn, avoiding hemolysis, and transferred to a clean centrifuge tube, allowed to coagulate, centrifuged within 10 minutes, and the serum removed.

The total calcium content is in the serum. On standing, the cells become permeable to calcium, thus lowering the result. The variation of cell volume in different bloods introduces an error in calcium determinations on whole blood.

Pipet 1 cc. serum or citrated plasma into a 15-cc. centrifuge tube, and while rotating the tube, slowly add 3% ammonium oxalate, equal in volume to approximately  $\frac{1}{2}$  the amount of serum or plasma. Mix thoroughly and allow to stand 30 minutes. Rub down walls of the tube with a rubber policeman (washing the policeman with a small amount of distilled water), and centrifuge until clear; usually 5 minutes is ample time. Completely remove the supernatant liquid by means of a siphon or by careful decantation, washing down the walls of the tube, using in all approximately 10 cc. 1-50 ammonia water. Centrifuge immediately, and completely remove the wash water. Repeat washing and centrifuge once more. Dissolve the precipitate in 5 cc. approximately normal  $\text{H}_2\text{SO}_4$  heated to  $75^\circ$  and titrate with 0.01 *N* K permanganate to the first faint pink, keeping it hot. One cubic centimeter 0.01 *N* potassium permanganate is equivalent to 0.2 mg. calcium.

**Barium** is precipitated as iodate and determined by the iodate-iodide reaction method. Ash sample, cover with concentrated HCl, carefully evaporate to dryness. To residue add 10 cc. slightly alkaline water; add *N*/6 solution of  $\text{KIO}_3$  to amount of 5 cc. excess, stirring continuously and for a minute after completion of addition. Let stand for 5 minutes; centrifuge. Wash precipitate with ammonia water and with 95% alcohol. Wash precipitate into 25-cc. separatory funnel with water, treat with 1 cc. 10% KI solution and 1 cc. concentrated  $\text{H}_3\text{PO}_4$ . Let stand for 5 minutes and fill to 10-cc. mark, and determine iodine:



Hill and Zink: *J. Am. Chem. Soc.* 31:43 (1909).

**Aluminum.** Ash 2 cc. blood at a low red heat in a platinum or silica dish. Dissolve ash in 5 cc. 10%  $\text{H}_2\text{SO}_4$ , make up to volume in a 50-cc. volumetric flask. Transfer an aliquot to a 10-cc. centrifuge tube, add 1 cc. 60%  $\text{NH}_4\text{CNS}$ . The ferric thiocyanate is shaken out with ether 4-5 extractions removed by suction. The clear solution is placed in a 25-cc. volumetric flask, and 2.5 cc. of a mixture of glycerol

and 10% citric acid (4:1) are added. The solution is neutralized with  $\text{NH}_3$  gas. One-half cubic centimeter mono-sodium alizarin sulfonate is added and the pH adjusted to 3.6 or less with acetic acid. The solution in 24 hrs. is compared against a standard in a colorimeter.

Underhill and Peterman: *Am. J. Physiol.* 90:1 (1929) for details.

**Fluorine.** One-half gram alizarin is dissolved in 200 cc. alcohol; 1.5 g.  $\text{ZrCl}_4$  is dissolved in 75 cc. alcohol. Mix, and allow red precipitate to settle, centrifuge and decant; wash precipitate with alcohol. The precipitate is then made to a volume of 25 cc. with alcohol, and 5 cc. is then added to 100 cc. water and shaken. This serves as the indicator. To 0.5 cc. of neutral or somewhat acid solution to be tested, add 0.5 cc. of concentrated HCl and then 0.2 cc. of the indicator. Immediate color change will occur in test solutions with more than 0.3 mg. fluorine per cubic centimeter. Smaller concentrations will bring about color change before expiration of 15 seconds. Sodium sulfite solution added before acidification would eliminate interference of sulfate, oxalate, or phosphate. A positive test is obtained also with complex ions containing fluorine.

Armstrong: Thesis, Univ. of Minn., Minneapolis, U. S. A. (1932); *Proc. Soc. Exptl. Biol. Med.* 29:414 (1932).

**Plasma Chlorides** (Kok, Cavett and Holdridge).  $\text{HgCl}_2$  is very slightly dissociated. As standard  $\text{Hg}(\text{NO}_3)_2$  solution is added, the  $\text{Cl}^-$  ion unites with  $\text{Hg}^{++}$  forming  $\text{HgCl}_2$ . On addition of excess  $\text{Hg}^{++}$  ions the mercury forms a precipitate with the indicator, sodium nitroprusside, thus producing a turbidity for an end-point. Chlorides are run on plasma instead of blood because the corpuscles contain about one-half as much chloride per volume as the plasma; thus a variation in total corpuscle volume would give false values (as high as 15% variation in anemia).

**Reagents.** Standard  $\text{Hg}(\text{NO}_3)_2$  solution (1 cc. is equivalent to 1 mg. NaCl). Dissolve 1.853 g. of red mercuric oxide in an excess of concentrated nitric acid and dilute the solution to 1 liter. The solution may be checked by titrating against a standard NaCl solution.

5% sodium nitroprusside

$\frac{2}{3}$  N  $\text{H}_2\text{SO}_4$

10% sodium tungstate (chloride free)

The blood is drawn with the same technique as for  $\text{NaHCO}_3$  thus preventing the chlorine shift which would increase plasma chlorides.

**Method.** A Folin-Wu filtrate of the blood or plasma is prepared. (2 cc. of blood or plasma, 14 cc. of water, 2 cc. of  $\frac{2}{3}$  N  $\text{H}_2\text{SO}_4$  and 2 cc. of 10% sodium tungstate are thoroughly shaken together and filtered or centrifuged.)

Five cubic centimeters of the filtrate are pipetted into a large test tube and 3 drops of fresh 5% sodium nitroprusside solution are added. Mercuric nitrate (1 cc. is equivalent to 1 mg. NaCl) is added from a microburet until a permanent turbidity is produced on the addition of one drop.

(Titration value in cc.  $- X$ )  $\times 200$  = mg. of NaCl per 100 cc. of plasma.

TABLE I

5 cc. of Folin-Wu Filtrate		10 cc. of Folin-Wu Filtrate	
Titration value in cc.	Titration correction $X$	Titration value in cc.	Titration correction $X$
1.5	0.07	3	0.14
2.0	0.08	4	0.16
2.5	0.09	5	0.18
3.0	0.10	6	0.20
3.5	0.11	7	0.22

Should 10 cc. of the filtrate be titrated the formula becomes (titration value in cc.  $- X$ )  $\times 100$  = mg. NaCl per 100 cc. of blood or plasma.

Kok: Arch. Neerl. Physiol. 16:132 (1931).

Cavett and Holdridge: J. Lab. Clin. Med. 18:944 (1933).

Votoček: Chem. Ztg. 42:257, 271 (1918).

**Bromine.** The material to be used is incinerated and the ash extracted with dilute HCl. The extract is treated with 10%  $\text{KHSO}_4$  and  $\text{KMnO}_4$ . The free bromine liberated is transferred by means of a current of air into a flask containing a definite volume of fuchsin  $\text{H}_2\text{SO}_3$  and the bromine content determined colorimetrically by means of the violet color produced with the standard solution of bromine.

Wünsche: Arch. exptl. Path. Pharmacol. 84:328 (1919).

**Inorganic Sulfate.** One cubic centimeter serum, urine, or body fluid is placed with an equal quantity of distilled water in a 5-cc. centrifuge tube, 1 cc. 20% trichloroacetic acid is added, and the volume made up to 5 cc. with distilled water. After mixing and centrifuging, 0.5 cc. of supernatant fluid is withdrawn and added to a second centrifuge tube containing 1 cc.  $\frac{1}{2}\%$  benzidine in acetone. The tube is capped (to prevent loss of acetone) and after standing 30 minutes is centrifuged for 30 minutes. The supernatant fluid is poured off and the tube inverted on filter paper for 3 minutes. After drying the mouth of the tube the precipitate is washed with 5 cc. acetone, stirred up, and recentrifuged for 15 minutes. After being inverted for 5 minutes the mouth of the tube is dried and  $\frac{1}{2}$  cc. of 0.2 N HCl

is added. The precipitate may be dissolved with the aid of gentle heat but not allowed to boil. After cooling, 2 cc. of water,  $\frac{1}{2}$  cc. dilute  $H_2O_2$ , and  $\frac{1}{2}$  cc. fresh  $\frac{1}{2}\%$   $FeCl_3$  solution are added, the contents mixed, and the tube allowed to stand for 5 minutes. After about 5 minutes the color is fully developed, and it remains constant for about 10 minutes. Standards should be prepared simultaneously with the unknowns and have the same acidity.

Wakefield: J. Biol. Chem. 81:713 (1929).

**Preparation of Urine (Removal of Phosphates).** Transfer to a 100-cc. volumetric flask enough urine (usually 10–20 cc.) to contain about 10–20 mg. of sulfur as sulfate. Dilute to about 50 cc. with water. Add one drop of phenolphthalein solution and then concentrated ammonium hydroxide drop by drop to a faint pink color. Add 10 cc. of 5% ammonium chloride and about 1.5 g. finely powdered magnesium carbonate. Make to mark, mix thoroughly by shaking for 1 minute, and let stand for 30 minutes. Filter, using a dry filter, into a dry flask. This filtrate is used for the following determinations of total sulfate and total sulfur.

**Total Sulfate (*Inorganic and Ethereal*).** To 1 cc. of filtrate in a 10-cc. crucible add 1 cc. of approximately *N* HCl. Heat on a water bath to dryness and for 10 minutes longer. Transfer to a Pyrex tube using 2 (1-cc.) portions of water, add 1 cc. of benzidine solution and proceed as in method for inorganic sulfate.

Fiske: J. Biol. Chem. 47:59 (1921).

**Ethereal Sulfate.** Subtract inorganic from total sulfate. The difference is ethereal sulfate.

**Total Sulfur.** Transfer approximately 0.1 cc. of Benedict's sulfur reagent (20 g. of crystalline copper nitrate and 5 g. of potassium chlorate in 100 cc.) and 1 cc. of filtrate to a small evaporating dish (6 cm.). Evaporate carefully to dryness on wire gauze or hot plate. Increase heat gradually, finally igniting at red heat for 2 minutes over free flame. Let cool for 5 minutes. Add 1 cc. *N* HCl and evaporate to dryness at low heat. The mixture will turn from green to brown. Transfer to a test tube with the aid of two 1-cc. portions of water. Add 1 drop of *N* HCl and 1 cc. of benzidine solution. Complete the determination as in the methods above.

Folin: Laboratory Manual of Physiological Chemistry, fourth edition.

**Neutral Sulfur.** Subtract from the total sulfur the total sulfate as determined above. The difference is neutral or unoxidized sulfur.

Hubbard: Proc. Am. Soc. Biol. Chem. J. Biol. Chem. 74:V (1927).

Wakefield: J. Biol. Chem. 81:713 (1929).

Fiske: J. Biol. Chem. 47:59 (1921).

**Volumetric Determination of Sulfur.** The sulfates of the urine are precipitated by means of benzidine solution, the precipitate being filtered off, and the sulfuric acid liberated from the salt of the weak base is titrated with 0.02 *N* NaOH, phenol red being used as an indicator.

*Benzidine Solution* is made by shaking 4 g. benzidine with about 150 cc. of water and 50 cc. of approximately *N* HCl in a 250-cc. flask. After all benzidine has dissolved, dilute to mark and mix.

*Removal of Phosphate.* [See preparation of urine in the preceding method.] This step is necessary for the highest accuracy, especially where the proportion of phosphorus to sulfur may be high as in half hourly specimens of urine. For 24-hour specimens where the highest accuracy is not desired it may be omitted.

*Inorganic Sulfate.* Pipet 5 cc. of the filtrate into a Pyrex test tube. Add 2 drops of a 0.04 per cent alcoholic solution of brom phenol blue and 5 cc. of water. Add approximately *N* HCl until the last trace of blue disappears and the solution is yellow. Add 2 cc. of benzidine solution. Let stand for 2 minutes. Add 4 cc. of 95 per cent acetone and let stand for 10 minutes. Prepare a thin mat of paper pulp in a filtration tube. This mat should first be washed with water and then sucked dry. Filter off benzidine sulfate with very gentle suction. Wash down the sides of the test tube with 1 cc. 95 per cent acetone, transferring to filtration tube. Wash twice more with 1 cc. and finally with 5 cc. of acetone. Add about 2 cc. of water and poke the mat with a wire out through the bottom of the tube into the Pyrex test tube, rinsing the wire with a few drops of water. Add 2-4 drops of 0.05 per cent water solution of phenol red. Titrate with 0.02 *N* NaOH (prepared from 0.1 *N* NaOH by dilution), the solution being kept hot. At the beginning of the titration the filter tube is kept suspended in the mouth of the test tube and the alkali run through the filter tube to dissolve adherent sulfate. Rinse inner tube with 2-3 cc. of water, heat solution to boiling so that tube is further washed with condensed steam, and finally rinse inner tube with a few cubic centimeters more of water and remove. Titrate to a definite pink color that remains after boiling.

*Total Sulfate (Inorganic and Ethereal).* To 5 cc. of filtrate in a 100-cc. beaker add 1 cc. of approximately 3 *N* HCl. Heat on a water bath to dryness and for 10 minutes longer. Transfer to a Pyrex tube using five 2-cc. portions of water, add 2 cc. of benzidine solution and proceed as in method for inorganic sulfate.

*Ethereal Sulfate.* Subtract inorganic from total sulfate. The difference is ethereal sulfate.

*Total Sulfur.* Transfer approximately 0.25 cc. of Benedict's sulfur reagent (20 g. crystalline copper nitrate and 5 g. of potassium

chlorate in 100 cc.) and 5 cc. of filtrate to a small evaporating dish (6 cm.). Evaporate carefully to dryness on wire gauze or hot plate. increase heat gradually, finally igniting at red heat for 2 minutes over free flame. Let cool for 5 minutes. Add 1 cc. 3*N* HCl and evaporate to dryness at low heat. The mixture will turn from green to brown. Transfer to a Pyrex tube with the aid of five 2-cc. portions of water. Add 1 drop of *N* HCl and 2 cc. of benzidine solution. Complete the determination as in the methods above but use in place of the first 1-cc. portion of acetone in washing 2 cc. of 50% acetone.

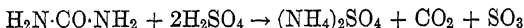
*Neutral Sulfur.* Subtract from the total sulfur the total sulfate as determined above. The difference is neutral or unoxidized sulfur.

**Nitrates and Nitrites.** To 5 cc. toluene-free urine add 5 cc. mercuric chloride solution. Dilute and filter, diluting the filtrate to suit purpose, or take 1 cc. of plasma or serum in a 100-cc. volumetric flask, add water to 6 cc. and add 1 cc. 5% mercuric chloride solution. Make to volume and shake. If whole blood is used, add 1 cc. 1% sodium carbonate before making up to volume. Make a suitable dilution of this. Using two test tubes with glass stoppers, pipet 1 cc. diluted blood-filtrate or urine-filtrate into one of the tubes and 1 cc. of standard potassium nitrate solution containing 0.0005 mg. nitrate-N in the other. These tubes are placed in an ice and salt freezing mixture and 10 cc. of the diluted reagent (20 cc. H<sub>2</sub>SO<sub>4</sub> + 5 mg. diphenyl benzidine + 5 mg. NaCl) added slowly down the side of the tube. Mix gently and add 10 cc. concentrated sulfuric acid slowly with pipet so as to form a layer at the bottom of the tube, keeping the tip of the pipet beneath the surface of the reagent and acid solutions. Mix the solutions gently so as not to cause a rise in temperature, and after cooling, place, with occasional stirring, in a water bath at 50° for 5 minutes. Remove the tubes and let them stand for 1½ hours. Then compare in the colorimeter.

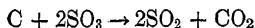
Whelan: J. Biol. Chem. 86:189 (1930).

**Nitrogen** (Micro-Kjeldahl method). The various nitrogenous compounds, with a few exceptions, are broken down by heating with concentrated sulfuric acid, the nitrogen being converted into ammonia, and the carbon into carbon dioxide. The ammonia is retained in the solution as (NH<sub>4</sub>)HSO<sub>4</sub>. It is then liberated by the addition of sodium hydroxide and distilled into hydrochloric acid and determined by Nesslerization.

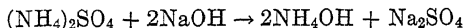
Urea is decomposed as follows:



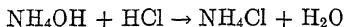
Carbon resulting from the action of sulfuric acid on organic matter is oxidized as follows:



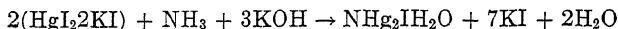
The ammonia is freed before distillation as follows:



During distillation the ammonia is received into HCl solution.



The Nessler reaction is



$\text{NHg}_2\text{IH}_2\text{O}$  crystallizes in red needles and may be determined gravimetrically if a sufficiently concentrated solution of ammonia is used. Since the colorimeter is adapted to micro-methods, it is desirable to keep the red substance in solution. This may be done by sufficient dilution of the sample and reduction of the alkalinity of Nessler's reagent. The reduction of alkalinity impairs the stoichiometric exactness of the method, and the value of the unknown and the standard should be of somewhat the same value for very accurate work.

**Phosphoric-Sulfuric Acid- $\text{CuSO}_4$  Mixture.** In order to increase the rate of oxidation, the boiling-point of  $\text{H}_2\text{SO}_4$  is raised by the addition of  $\text{H}_3\text{PO}_4$ . To 50 cc. of a 5%  $\text{CuSO}_4$  solution add 300 cc. of 85% phosphoric acid and mix. Add 100 cc. concentrated  $\text{H}_2\text{SO}_4$  (free from  $\text{NH}_3$ ), mix, and cover well to prevent absorption of  $\text{NH}_3$  from the air. Dilute 50 cc. of the acid mixture with 50 cc. of water and keep well protected to prevent the absorption of  $\text{NH}_3$  from the air.

**Hydrochloric acid (0.1 N).** For receiving the  $\text{NH}_3$  when it is distilled over.

**Sodium hydroxide (15 N).** For freeing ammonia from the ammonium sulfate. Rotate 1 kg. NaOH and 1 liter  $\text{H}_2\text{O}$  in a 2-liter balloon flask until dissolved. Transfer to a bottle it just fills, wrap in blanket and allow carbonate to settle.

**Nessler's reagent** (a solution of the double iodide of mercury and potassium ( $\text{HgI}_2\cdot 2\text{KI}$ ) containing sodium hydroxide). Transfer 150 g. KI and 110 g.  $\text{I}_2$  to a 500-cc. Florence flask. Add 100 cc.  $\text{H}_2\text{O}$  and an excess of metallic mercury (140–150 g.). Shake the flask continuously for 7–15 minutes or until the dissolved  $\text{I}_2$  has nearly all disappeared. The solution becomes hot. As soon as the red  $\text{I}_2$  solution has begun to become paler, though still red, cool rapidly in running water but continue the shaking until the reddish color of the  $\text{I}_2$  has been replaced by the greenish color of the double iodide. Separate the solution from the surplus mercury by decantation and wash with distilled water. Dilute the solution and washings to a volume of 2 liters. From the 15 N NaOH solution made by dissolving NaOH in an equal weight of water and allowing the carbonate to precipitate out, decant the clear supernatant liquid and dilute to 5 volumes.

Titrate and adjust to 2.5 *N*. Introduce into a large bottle 3.5 liters of 2.5 *N* NaOH solution, add 750 cc. of the double iodide solution and 750 cc. distilled water, giving 5 liters of Nessler's solution.

*Standard Ammonium Sulfate Solution:* For use for comparison with the unknown. (Stock solution contains 0.1 mg. of nitrogen per cc.) Dry the crystals of  $(\text{NH}_4)_2\text{SO}_4$  at 100°. Weigh out 0.4719 g., dissolve in distilled water, and make up to 1 liter. Add 1 cc. to 24 cc. of water to prepare standard for Nesslerization.

*Procedure.* Introduce 1 cc. of urine into a 100-cc. volumetric flask, fill with  $\text{H}_2\text{O}$ , and mix. Transfer 1 cc. of the diluted urine into a Pyrex test tube previously heated until the bottom is dry inside, to reduce the danger of bumping. Add 1 cc. of the diluted acid mixture. Boil vigorously over a micro-burner and also heat sides of tube until fumes of  $\text{SO}_3$  begin to fill the tube. When the test tube is nearly full of fumes, reduce the flame to 2 mm. Cover the mouth of the test tube with a watch glass. Continue the heating for 2 minutes, counting from time the test tube became filled with fumes. The solution will turn yellowish or darken and then become colorless or greenish. The heating must be continued for double the time required for the solution to turn colorless or greenish. Remove the flame and allow the digestion-mixture to cool for 1 or 2 minutes. If digestion bumps, put in 3 grains of sand. Zinc should not be put in the digestion to prevent bumping as the copper plates out on it, it dissolves too fast, and is liable to spray over during the distillation enough to make the Nessler solution cloudy. Another source of clouding is tap water in the Nesslerization. Some beginners mistake steam for  $\text{SO}_3$  fumes and do not boil off all the water. Since only 1 cc. of the diluted acid mixture is used, it will be  $\frac{1}{2}$  cc. when the water is boiled out and hence one can tell roughly when the fumes of  $\text{SO}_3$  should appear. There will be drops of condensed water inside the test tube which run back in the acid and lower its boiling temperature if the watch glass is put on too soon or the sides not heated. Most beginners allow  $\text{SO}_3$  to escape by having the flame high for too long a time. These fumes can be detected by the nose or by the coughing that they cause. When considerable  $\text{H}_2\text{SO}_4$  is decomposed by heat and allowed to escape as  $\text{SO}_3$  the test tube will dissolve and make the acid cloudy. If all the acid is boiled off, the  $(\text{NH}_4)_2\text{SO}_4$  will decompose. To prevent bumping place a very small U-tube with open end downward in distilling tube and add 5 cc.  $\text{H}_2\text{O}$  (or transfer to a dry Pyrex test tube, washing with 5 cc. or more of  $\text{H}_2\text{O}$ ) and connect with the micro-distilling apparatus fig. 56. Place 5 cc. of 0.1 *N* HCl in a similar test tube for a receiver. Add 2 cc. of 15 *N* NaOH to the digest so that it flows down the inside of the test tube and forms a layer beneath the acid, then stopper immediately, and distil  $\frac{1}{3}$  volume, or more if it

does not start to bump. Lower the receiver until the condenser is out of the acid and boil the digest so as to wash out condenser with

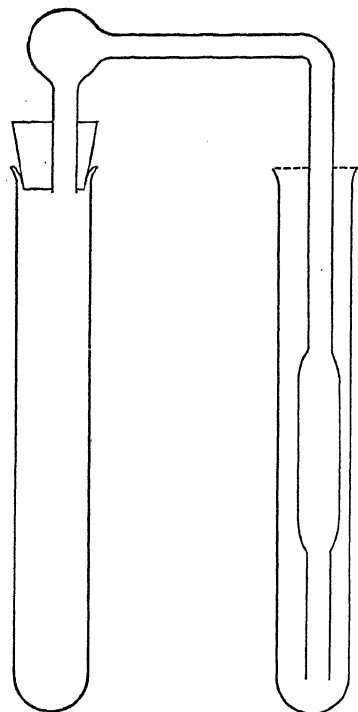


FIG. 56. Micro Kjeldahl distilling apparatus.

steam. Wash the outside of the condenser with wash bottle. Fill to 25-cc. mark with distilled water. Add 5 cc. of Nessler's solution, one-third at a time, while stirring. Add 5 cc. of Nessler's solution to 25 cc. of the standard ammonium sulfate solution. This will contain 0.1 mg. nitrogen. The unknown and the standard should be Nesslerized simultaneously and compared in the colorimeter within 10 minutes.

Caniff: Proc. Soc. Exptl. Biol. Med. 28:348 (1930).

Folin: J. Biol. Chem. 21:195 (1915).

—: J. Biol. Chem. 38:461 (1919).

Frederick: Analyst 50:183 (1925).

Richmond: Analyst 50:67 (1925).

**Acid-Soluble Phosphate in Blood.** Hydroquinone does not reduce  $\text{MoO}_3$  but does reduce phosphomolybdic acid.

*Reagents:* 20% trichloroacetic acid. Standard phosphate solution: Dissolve 0.11 g. highest purity dry  $\text{KH}_2\text{PO}_4$  in  $\text{H}_2\text{O}$  and

make up to 100 cc. This stock standard contains 0.25 mg. phosphorus per cc. It must be diluted 10 times before use. It should be preserved with chloroform. Benedict's hydroquinone-bisulfite reagent: Dissolve 30 g.  $\text{NaHSO}_3$  and 1 g. hydroquinone in  $\text{H}_2\text{O}$  and make up to 200 cc. Benedict's molybdic reagent: Dissolve 20 g. molybdic acid in 25 cc. 5 *N*  $\text{NaOH}$  with warming. Dilute to 200 cc. and filter if necessary. Transfer to a liter flask and add with constant agitation under cold running water an equal volume (about 200 cc.) of concentrated  $\text{H}_2\text{SO}_4$ .

The blood should be drawn, avoiding hemolysis, and transferred to a clean centrifuge tube, allowed to coagulate, centrifuged within 10 minutes, and the serum removed.

Hemolysis increases the acid-soluble phosphorus. The cells contain organic-acid-soluble phosphorus. If they are allowed to remain with the serum, during the first 2 or 3 hours the acid-insoluble phosphorus will increase at the expense of the acid-soluble fraction, thus giving low values. On longer standing (together), the inorganic fraction becomes high at the expense of the organic.

Transfer 1 cc. serum of plasma to a test tube, add 2 cc. water and 2 cc. 20% trichloroacetic acid. Shake vigorously, and after 10 minutes centrifuge. Transfer 3 cc. of the filtrate to a narrow test tube and add 3 cc. water. To a similar tube add 1 cc. of the diluted phosphate standard, containing 0.025 mg. phosphorus, and dilute with 5 cc. water. Now add to each tube 1 cc. Benedict's hydroquinone-bisulfite reagent and 1 cc. molybdic acid reagent. Loosely stopper the tubes and heat in a boiling water bath for 10 minutes. A blue color forms. Cool and compare in colorimeter.

Bell and Doisy: *J. Biol. Chem.* 44:55 (1920).

**Lead, Pb, in Tissues.** Weigh 25 g. of the tissue to be analyzed, free it from water by baking in a porcelain crucible, and then ash at a dull red heat. Cool the material and extract with as little dilute HCl and hot water as possible. All the ash must be dissolved, and after the first extraction it may be necessary to re-ash and treat with a solution of dilute HCl and tartaric acid. Neutralize the strongly acid solution with sodium hydroxide; then add 2 drops of methyl orange and make just acid with concentrated HCl. Transfer quantitatively to an Erlenmeyer flask and pass hydrogen sulfide through the solution for 15 minutes, shaking every 2 minutes. Stopper the flask tightly and let stand 24 hours. Filter and immediately wash the precipitate with 50 cc. pure warm water. Dissolve the precipitate in as little dilute  $\text{HNO}_3$  as possible (2-5 cc.) and boil for 3 minutes to expel hydrogen sulfide. Cool and neutralize with sodium hydroxide. Make just acid with acetic acid and add 3 drops of a saturated solution of potassium chromate. Boil for 5 minutes, stopper tightly, and allow the solution to stand 24 hours. The extremely small amounts of lead chromate will then be separated. Filter and wash the precipitate with 30 cc. hot distilled water. With as little hot distilled water as possible, wash all traces of the precipitate into a 200-cc. beaker and add just enough dilute HCl to completely dissolve the precipitate with constant stirring. Immediately add an excess of potassium iodide solution and determine the free iodine with a colorimeter or by titration with 0.005 *N* thiosulfate; 1 mg.  $\text{I}_2$  = 0.544 mg. lead.

**Lead in Urine.** Urine is made strongly alkaline with ammonium hydroxide, and allowed to stand until precipitation of basic phosphates has left a clear supernatant liquid (1-24 hours). The latter is

decanted and discarded, and the remainder is filtered with suction on a Büchner funnel. The filter paper and precipitate are ashed, and the ash is treated as described above.

Fairhall: *J. Ind. Hyg.* 4:9 (1922).

**Arsenic.** Organic matter in 10 g. dried tissue is destroyed by wet combustion in 16-cm. evaporating dishes covered with watch glasses which fit inside the dish. The digestion is started on the water bath with nitric acid, the sample being moistened with water to prevent ignition. When largely liquefied, 10 cc.  $\text{H}_2\text{SO}_4$  is added and the digestion continued, first on the water bath and later in an oven at  $200^\circ$ . Nitric acid is added as needed to keep the space beneath the cover glass continuously filled with brown fumes. When the digestion is complete, the cover glass is removed and the nitric acid eliminated by repeated additions of water, and heating until a drop of the solution gives no color with diphenylamine reagent.

Prior to introduction into the Marsh-apparatus the solution is diluted to about 25 cc., heated to boiling, and a small crystal of stannous chloride added to insure that the arsenic is in the trivalent state. The hydrogen generator is started with sufficient 1:4  $\text{H}_2\text{SO}_4$  to cover the zinc, and allowed to run until the action has died down to a practically constant rate and the flame is between 1 and 2 mm. in size, after which the sample is run in at such a rate as not appreciably to increase the flow of gas. Marshing is continued for half an hour, after the last of the sample has been introduced.

The mirrors are dissolved in standard iodine solution. The portion of the tube containing the mirror is cut off, dropped into a glass-stoppered 100-cc. flask, and 25 cc. 0.002 *N* iodine solution added, followed by 2 cc. 1% sodium bicarbonate. The stopper is then inserted, covered with a few drops of KI solution, and the neck of the flask covered with an inverted test tube to prevent evaporation of the sealing liquid. Solution of such large mirrors as those obtained is very slow, requiring sometimes as long as 24 hours. After solution is complete the stopper and neck of the flask are rinsed, and the excess of iodine is determined in a colorimeter or by titration with 0.002 *N* thiosulfate solution.

Remington: *J. Am. Chem. Soc.* 49:1410 (1927).

**Manganese.** Weigh 10 g. of the sample into a silica dish, char over a low flame and then ash in the muffle at  $500^\circ$  for 3–4 hours. Cool, moisten with water and then add 1 cc. concentrated  $\text{HNO}_3$ . Evaporate to dryness on the hot plate and return to muffle, this time at a temperature of  $400^\circ$ . Ashing is usually complete after this one application of  $\text{HNO}_3$ . Cool, add 15 cc. 1–1  $\text{HCl}$ , and heat to dissolve ash. Then add 10 drops 30%  $\text{H}_2\text{O}_2$  and heat until oxygen ceases to

be liberated. If convenient, allow to stand overnight, then transfer to a 100-cc. volumetric flask and dilute to the mark. Fifty cubic centimeters of this ash solution are pipetted into a small beaker; 1.5 cc. dilute (1-3)  $\text{H}_2\text{SO}_4$  is added and the mixture is evaporated to dryness on the hot plate. The temperature is increased and the  $\text{H}_2\text{SO}_4$  is driven off, finishing up with gentle ignition in the muffle. Then add 1 cc. dilute  $\text{H}_2\text{SO}_4$  and 1 cc. syrupy  $\text{H}_3\text{PO}_4$  and 25 cc. water. Evaporate to about 10 cc. when all the salts should be in solution. Add 0.3 g. potassium periodate, cover beakers with watch glasses, and heat at just under boiling temperature for 30 minutes. Transfer to a 25-cc. volumetric flask, dilute almost to mark, and heat 15 minutes longer. Cool, make up to volume, and compare in colorimeter with a standard made up in the same manner.

Remington: Personal communication.

**Iron, Fe.** Ash as for manganese. Ten cubic centimeters of the ash solution is pipetted into a 50-cc. volumetric flask; 2.5 cc. concentrated  $\text{HCl}$  (making the total amount of acid approximately 3 cc.) is added and the solution is diluted to the mark. From this solution 5 cc. is pipetted into a test tube. One cubic centimeter of 50%  $\text{KCNS}$  and 5 cc. amyl alcohol are added and the mixture thoroughly shaken. The colored layer of amyl alcohol is then compared in a colorimeter with a standard prepared in the same manner. The acidity and concentration of the standard and the unknown must be approximately the same. It is best to run a preliminary test to determine the standard to be used. (Make standard solutions containing 0.01-0.04 mg. iron for comparison.)

Remington: Personal communication.

**Copper.** Ash as for manganese. Ten cubic centimeters of the ash solution is pipetted into a 25-cc. flask and heated in a water bath to a temperature of 40-50°. Neutralize with concentrated  $\text{NH}_4\text{OH}$  to a distinct red color with phenolphthalein, adding the drop of indicator after the first permanent appearance of precipitate. Then add 1 cc. glacial acetic acid and return to water bath for a few minutes longer. Remove from bath, allow solution to come to room temperature, and then add, in order, 1 cc. 10%  $\text{KCNS}$  and 10 drops pyridine (re-distilled), with slight shaking after each addition. Finally 5 cc. of  $\text{CCl}_4$  accurately measured is added and the volume made up with water to approximately 25 cc. After thorough shaking the  $\text{CCl}_4$  has taken up the green-colored copper thiocyanate pyridine compound, the water portion is mostly removed, and the  $\text{CCl}_4$  portion used for the colorimetric comparison.

Remington: Personal communication.

**Methyl Alcohol.** Dissolve 0.1 g. fuchsin crystals in 88 cc. water and add 0.7 g.  $\text{NaHSO}_3$ . After standing 1 hour add 25 drops concentrated  $\text{HCl}$ .

If the sample contains more than 60% alcohol, use 10 cc. and dilute with 20 cc. water; if 20–60% use 10 cc.; if less than 20% use a 20-cc. aliquot. Introduce part of sample into a 50-cc. flask provided with a small glass tube 75 cm. long, bent twice nearly at right angles, which serves as a condenser. If the sample is acid, neutralize it with 0.1–0.5 g. precipitated calcium carbonate. By means of a small flame distil 1 cc. into a small test tube cooled by ice-water. The last vertical column of the tube should not become heated. Place 0.1, 0.2, and 0.3 cc. distillate separately into three tubes, fill each to 5 cc. with water, add 0.4 cc. of 50% sulfuric acid and 5 cc. of 1% potassium permanganate solution. Allow the mixture to stand 2 minutes. Decolorize by adding 1 cc. 8% oxalic acid, followed by 1 cc. concentrated sulfuric acid. Then add 5 cc. fuchsin-sulfurous acid solution and mix. If methyl alcohol is present a violet or reddish purple color is developed in 2 hours. Compare with standard run through the analysis simultaneously.

Kimugasa, Hattori, and Akiyama: J. Pharm. Soc. Japan 48:767 (1928).

**Formaldehyde in Green Leaves.** Steam-distil the leaves, evaporate the distillate with ammonia (forming urotropine), and add bromine water to residue, converting it to the tetra-bromo derivative (Usher and Priestly). Or, inject with capillary tube a concentrated solution of sodium hydrogen sulfite containing an excess of *p*-methyl-amino-*m*-cresol, into leaf of *Agave mexicana* or other suitable plant. Dip leaf in absolute alcohol; red precipitate on exposure to light indicates presence of formaldehyde (Kimpflin).

Kimpflin: Compt. rend. Acad. Sci. 144:148 (1907).

Usher and Priestly: Proc. Roy. Soc. London 77:369 (1906).

**Acetaldehyde in Cerebrospinal Fluid.** The fluid is collected by lumbar or sub-occipital tapping, measured, then poured into a small distilling apparatus and neutralized with phosphoric acid. Add 3.5 cc. aldehyde-free alcohol and distil to mark into a 7-cc. graduated test tube containing 0.5 cc. water and packed in ice. The 7 cc. of distillate are added to 3 cc. freshly prepared 10% *m*-phenylenediamine. Add 7 cc. standard acetaldehyde to 3 cc. reagent and mix. Let unknown and standard stand for 20 minutes in darkness; then compare red color in colorimeter.

Compt. rend. soc. biol. 96:1042 (1927).

**Glyoxal.** To 10 cc. of solution to be tested are added, in order, 2 cc. arsenophosphotungstic acid reagent, 1 cc. *M*  $\text{NaCN}$ , and 5 cc. *M*

$\text{Na}_2\text{CO}_3$ ; after standing 10 minutes the mixture is diluted to 100 cc. with water and the depth of the color read in a colorimeter against a standard in which 10 cc. 0.001 *M* glyoxal solution and the same amount of reagents as mentioned above are treated in the same manner. Unless the concentration of glyoxal in the solution to be analyzed is approximately known, it is necessary to make a preliminary determination for orientation; another portion of the solution is then diluted or the standard diluted, so that approximately equal amounts of standard and unknown may be taken for the determination.

Ariyama: J. Biol. Chem. 77:359 (1928).

**Furfural.** To 25 cc. unknown water-solution add 25 cc. absolute alcohol. Then place the tube in a water bath at 15° and allow it to stand until the solution is of the same temperature as the bath. Add 2 cc. aniline and 0.5 cc. HCl, producing a red color. Allow tubes to stand at 15° for 15 minutes and read in colorimeter against a standard solution.

Tolman: J. Am. Chem. Soc. 28:1619 (1906).

**Determination of Acetone Bodies in Urine.**  $\beta$ -Hydroxybutyric acid can be oxidized to acetoacetic acid with acid dichromate. Upon being heated, acetoacetic acid breaks down to form carbon dioxide and acetone. Into a 200-cc. Erlenmeyer flask are introduced 5 cc. of urine, 5 cc. of 40%  $\text{CuSO}_4$ , 5 cc. of a well-shaken 20% suspension of  $\text{Ca}(\text{OH})_2$ , and 5 cc. of water. The mixture is shaken very thoroughly and allowed to stand for  $\frac{1}{2}$  to  $\frac{3}{4}$  hour with occasional shaking. After filtration a 5-cc. aliquot (if a large amount of acetone is suspected, 1 cc. may be taken and 4 cc. of water added) is transferred to a micro-Kjeldahl containing a small U-tube with the open ends downward to prevent bumping. The Pyrex tube is closed with a 2-hole rubber stopper fitted with the usual condenser tube, and with the tip of a buret. The connection between buret and tip should be clamped. The receiver tube is cooled with ice-water, and contains 0.5 cc. of water. The tip of the condenser should be below the surface of the water. One cubic centimeter of liquid is then distilled over slowly.

If it is desired to determine pre-formed acetone plus acetoacetic acid separately, the receiver tube should be removed at this point, and replaced by another one. Otherwise it is left in place.

The hydroxybutyric acid remaining is then oxidized by the slow addition, with continued boiling, of 2 to 3 cc. of 0.2%  $\text{K}_2\text{Cr}_2\text{O}_7$  in 50%  $\text{H}_2\text{SO}_4$ . Distillation is continued until the volume in the receiver is a little less than 5 cc.

Determination of acetone in the distillates is as follows: A standard solution is prepared by making 1 cc. of acetone (sp. gr. 0.792) to 100 cc., and then diluting 12.63 cc. of this solution to 1 liter. This stock

solution contains 0.1 mg. per cc. It will keep about one month. Before using, dilute 10 times. If great accuracy is desired, the strength of the original solution before dilution can be determined by adding excess standard iodine solution and determining the excess. Four test tubes are graduated at 5 cc. In one of them is placed the unknown. In the other three are placed respectively enough of the standard solution to correspond to 0.01, to 0.03, and 0.05 mg. acetone. All volumes are made up to 5 cc. To each tube are added 5 cc. of approximately 7.5 *N* NaOH and 0.25 cc. of salicylaldehyde, the contents are mixed thoroughly, and the tubes are set in boiling water for 5 minutes. The unknown is compared with the standard most nearly matching it.

If one does not desire to determine  $\beta$ -hydroxybutyric acid, the defecation of the urine sample is not necessary. In this case, the acetone may be distilled directly from 2 to 10 cc. of urine, and the determination run upon the distillate as above.

Behere and Benedict: *J. Biol. Chem.* 70:487 (1926).

**Carotin** is determined directly in the colorimeter. The standard is prepared as follows: In a 250-cc. flask put 20–30 g. butterfat and add 2 cc. aldehyde- and acetone-free 20% alcoholic KOH for each gram of fat. Reflux 1 hour. Dissolve the saponified substances in 3 volumes distilled water. Cool the solution and extract the pigments with 200 cc. ether in a separatory funnel. Draw off the soap solution and extract twice again with ether. Shaking carefully at first and more vigorously later, wash the extracts with water until the washings show no basic reaction with phenol red. Dry the ether-extract with neutral fused  $\text{CaCl}_2$  for several hours with occasional shaking. Decant or filter the extract into a 1-liter Claisen distilling flask and evaporate under reduced pressure at room temperature. Dissolve the residue in boiling 95% alcohol, precipitate the cholesterol by adding an excess of 1% solution of digitonin in 90% ethyl alcohol, allow to stand overnight, and filter. Remove the alcohol by evaporation under reduced pressure.

Palmer and Eckles: *J. Biol. Chem.* 17:191 (1914).

**Xanthophyll** is determined directly. The standard is made as follows: Into a 250-cc. flask add 100 cc. of pure acetone and a well-colored egg yolk and mix thoroughly. Reflux over a water bath for  $\frac{1}{2}$  hour. Filter through a hot-water-jacketed funnel to remove the coagulated protein, wash with a small amount of hot acetone, and collect washings and filtrate in a 250-cc. flask. Evaporate the acetone, add 50 cc. aldehyde- and acetone-free 20% methyl alcoholic KOH and reflux 1 hour. Dissolve the soap in 3 volumes water and

extract the pigment with ether. Evaporate the ether. Dissolve the residue in 100 cc. petroleum ether and extract the xanthophyll successively with 10 cc. 85%  $\text{CH}_3\text{OH}$  and 100 cc. 90%  $\text{CH}_3\text{OH}$ , and twice with 50 cc. 92%  $\text{CH}_3\text{OH}$ . Recover the xanthophyll by mixing the combined  $\text{CH}_3\text{OH}$  extract with 125 cc. ether while slowly adding distilled water; after the separation draw off the watery  $\text{CH}_3\text{OH}$ . The ether solution is washed with water. This ether solution of xanthophyll deteriorates rapidly.

Palmer: J. Biol. Chem. 23:261 (1915).

**Blood Lipids.** Place about 75 cc. of alcohol-ether mixture (3:1) into a 100-cc. volumetric flask and add with shaking 2 cc. of blood plasma or corpuscles. Immerse the flask in hot water, rotating it continuously until boiling begins. Cool and dilute to volume with alcohol-ether mixture. Allow the proteins to settle, or centrifuge in closed tubes and use for fatty acids, cholesterol, and lipid phosphorus.

**Fatty Acids.** Twenty cubic centimeters of alcohol-ether mixture (not blood filtrate) are measured into a 200-cc. Erlenmeyer flask for a blank determination. This is allowed to evaporate simultaneously with a like volume of the above-prepared blood filtrate after adding to each 0.5 cc. of distilled water. When the solutions are evaporated to approximately  $\frac{1}{2}$  volume, 0.1 cc. of saturated potassium hydroxide is added to each and the evaporation continued until the odor of alcohol has disappeared. This is very important as the quantitative determination of fatty acids depends upon their insolubility in water.

After the saponification is complete 1 drop of thymol blue is added, and while the flask is kept in motion, sufficient dilute sulfuric acid is added to change the color from blue through yellow to pale pink. If the soap is not completely dissolved a drop or two of distilled water is added. The fatty acid mixture is placed in a vacuum desiccator containing soda lime, overnight.

The potassium sulfate fatty acid mixture is dissolved in 15 cc. of distilled water at 60° and transferred to a 50-cc. graduated centrifuge tube; 10 cc. of hot benzene is then added to each flask, washing the bottom and sides with a rubber policeman, and the benzene is transferred to the centrifuge tube which contains the fatty acid mixture. The tube is stoppered and shaken vigorously for 1 minute and centrifuged. The benzene fatty acid layer is removed with a pipet. The extraction is repeated twice with 5 cc. of hot benzene. Traces of sulfuric acid are removed from the benzene fatty acid mixture by shaking it with freshly boiled distilled water cooled to 60° and centrifugation.

The hot benzene fatty acid mixture is removed from the water layer by means of a pipet and titrated with 0.02 *N* potassium hydroxide in aldehyde-free 97-99% alcohol. Owing to its instability the potassium hydroxide solution is prepared fresh and standardized against 0.02 *N* sulfuric acid for each titration. Phenolphthalein is used as indicator. The end-point is stable for about 3 minutes.

The normality factor of KOH  $\times$  cc. of KOH used for titration = millimols (*mM*) of fatty acid titrated.

$mM$  fatty acid titrated  $\times \frac{100}{0.4} = mM$  of fatty acid per 100 cc. of blood.

The last figure  $\times 277.2$  (average molecular weight of fatty acids in blood) = mg. of fatty acid per 100 cc. of blood.

Smith and Kik: J. Biol. Chem. 103:391 (1933).

**Cholesterol.** Evaporate 10 cc. of the alcohol-ether extract in a glass dish to dryness on a steam bath and remove instantly. The residue is extracted with 3 cc. of chloroform, allowing the chloroform to run down the side of the dish from a pipet. Pour the chloroform extract into a dry 10-cc. volumetric flask or cylinder, holding the lip of the dish against the flask while draining. Repeat twice using 2 cc. of chloroform each time. Introduce into a second dry 10-cc. flask, 5 cc. of a standard cholesterol solution (1 cc. contains 0.1 mg.). Add to each flask 2 cc. of acetic anhydride and 6 drops of concentrated sulfuric acid. Make up to 10 cc. with chloroform and mix. Compare in the colorimeter after 10 minutes. (All apparatus used must be free from water.)

**Lipid Phosphorus.** In a large test tube, graduated at 6 cc., place 10 cc. of the alcohol extract and a small glass bead. Evaporate to dryness on a steam bath. Add 1 cc. of a concentrated nitric sulfuric acid mixture (1:1). Digest over a low flame until white fumes appear. Remove from the flame and add a drop of 30% hydrogen peroxide and heat gently for a few minutes. Repeat addition of peroxide and heating if solution is not clear; cool, add 2 or 3 cc. of water and 0.75 g. of sodium carbonate, slowly. Dilute to 6 cc. A blank determination should be made on the reagents. The analysis for phosphorus is carried out in the same way as for blood phosphorus.

**Bile Salts.** The determination is based upon the color produced by the interaction of bile salts with furfural in a sulfuric acid solution. The color produced by the unknown sample is compared in a colorimeter with the color produced by a known amount of bile salt similarly and simultaneously treated. The color is due to the cholic acid part of the conjugated bile acid.

An approximately 0.9% solution of furfural is prepared by dissolving 9 cc. of thrice distilled furfural in a liter of water. The resulting solution should be clear and colorless.

Forty-five per cent (by volume)  $\text{H}_2\text{SO}_4$  is prepared by pouring 450 cc. of concentrated  $\text{H}_2\text{SO}_4$  into 550 cc. of distilled water, cooling the mixture under the tap.

Unknown and standard solutions of bile salt (either sodium glycocholate or sodium taurocholate). The commercial salts ordinarily obtained contain usually only about 50% by weight of bile salt. If such is used, the standard must be standardized with pure bile salt. The standard is made to contain 1 mg. of bile salt per cc. Preserve with chloroform and keep in ice box.

Transfer 1 cc. unknown to a 20-cc. test tube. In another tube place 1 cc. of the standard bile salt solution (either sodium taurocholate or sodium glycocholate), containing 1 mg. of the salt; to the standard and to the unknown add 1 cc. of 0.9% furfural and 6 cc. of 45%  $\text{H}_2\text{SO}_4$ . Mix at once and set in a water bath at 65° for 8 minutes. It is necessary that the temperature of the bath be not allowed to rise appreciably above 70° on account of the possibility of charring. After the color has been developed, remove the tubes and set them in a beaker of tap water for 5 minutes. Compare in colorimeter, using neon light passed through Corning glass H. R. red and heat-absorbing filters.

Gregory and Pascoe: J. Biol. Chem. 83:35 (1929).

**Surface Tension Test.** This test is based upon the principle that bile salts have the property of reducing the surface tension of fluids in which they are contained. The test is performed as follows: Cool about 10 cc. of urine in a test tube to 17° or lower, and sprinkle *a few particles* (not lumps) of finely pulverized sulfur upon the surface of the fluid. The presence of bile salts is indicated if the sulfur sinks to the bottom of the liquid, the rapidity with which the sulfur sinks depending upon the amount of bile salts present in the urine. The test is said to indicate the presence of bile salts when the latter are in the proportion of 1:120,000.

Allen has suggested the quantitative determination of bile salts by a surface tension method. Urine preserved with thymol may respond positively to this test.

Allen: J. Biol. Chem. 22:505 (1915).

**Homogentisic Acid.** Dilute 5 cc. alkapton-urine to 50 cc. in a 100-cc. volumetric flask. Add 10 cc. ammonium molybdate (in 5N  $\text{H}_2\text{SO}_4$ ) and an appropriate amount of hydroquinone standard with the same amounts of molybdate and 1%  $\text{KH}_2\text{PO}_4$  and fill to mark.

After 5 minutes compare in colorimeter. One milligram of hydroquinone gives the same color as 0.79 mg. homogentisic acid.

Briggs: J. Biol. Chem. 51:453 (1922).

**Phenol.** Transfer 20 cc. urine to a 100-cc. volumetric flask. Add 10 cc. of a 5% silver lactate solution in 5% lactic acid or until no more precipitate is obtained; then add a few drops of colloidal iron and shake. Fill to the mark with water, shake, and filter. Transfer 50 cc. of the filtrate to a 100-cc. volumetric flask, and to it add a sufficient quantity of saturated NaCl solution (containing 10 cc. strong HCl per liter) to precipitate all the silver. Fill to the mark with water and filter. Transfer 20 cc. of the filtrate to a large test tube, add 10 drops of concentrated HCl, and cover with a small funnel. Heat rapidly to boiling over a free flame and then place in boiling water bath (usually a tall beaker) for 10 minutes. Remove the tube, cool, and transfer to a 100-cc. volumetric flask. Add 10 cc. of the reagent (750 cc. water, 100 g. sodium tungstate, 20 g. phosphomolybdic acid, 50 cc.  $H_3PO_4$ , 100 cc. concentrated HCl, reflux 2 hours and dilute to 1 liter) and 25 cc. saturated sodium carbonate solution. Make up to volume, shake, and let stand for 20 minutes. Read against a standard of 5 cc. 0.01 N HCl containing 0.5 mg. phenol similarly treated.

Folin and Denis: J. Biol. Chem. 22:305 (1915).

Ehrlich: Biochem. Z. 79:232 (1917).

***p*-Cresol.** Six-tenths cubic centimeter of 1.1%  $Na_2CO_3$  is placed in the left cup of the colorimeter; 0.25 cc. of the reagent (*p*-diazobenzenesulfonic acid, see histidine) are pipeted into the same cup and mixed; 0.15 cc. of the unknown containing either tyrosine or *p*-cresol is mixed in the cup exactly 1 minute after the reagent. The cup is set at 20 mm. The standard of 0.1% *p*-cresol is treated likewise.

Hanke and Kossler: J. Biol. Chem. 50:246 (1922).

**Formic Acid.** Place 10 cc. oxalated blood in a 100-cc. volumetric flask, dilute to 50 cc., and mix. Add 5 g. picric acid and rotate until the acid sinks. Make volume up to 100 cc., shake vigorously, and filter into a 100-cc. graduate. Five cubic centimeters of mercuric chloride reagent (10% mercuric chloride, 10% sodium chloride, and 15% sodium acetate) are then added to 50 cc. of filtrate in a 200-cc. flask, heated on water bath 2 hours, and calomel determined as follows: Cool flask and add 10 cc. 25% HCl, 4 g. KI, and 10 cc. 0.1 N iodine solution. The flask is rotated until all calomel disappears and the excess  $I_2$  is determined with the colorimeter or by

titration with thiosulfate. Grams formic acid = cc. 0.1 *N* iodine  $\times$  0.0023 in the aliquot of filtrate taken.

F. De Eds: *J. Lab. Clin. Med.* 10:62 (1924).

***d*-Lactic Acid.** Transfer 10 cc. tungstic acid blood filtrate by means of a pipet into a centrifuge tube, add 1 cc. 10%  $\text{CuSO}_4$  and 2 cc. 10%  $\text{Ca(OH)}_2$ , shake well and allow to stand  $\frac{1}{2}$  hour. It is then centrifuged and the clear liquid is poured into a small funnel in which there has previously been placed a well-cleaned piece of washed cotton. Tube and funnel are rinsed. The funnel allows the clear liquid to be run into a 200-cc. Erlenmeyer flask. To this solution which contains lactic acid, add 5 cc. bichromate solution (7.6234 g. per liter; 1 cc. = 3.5 mg. lactic acid), and 5 cc. concentrated  $\text{H}_2\text{SO}_4$  must now be added in drops. The flask is closed tightly with a rubber stopper and is placed in a water bath with temperature at  $60^\circ$  for approximately 50 minutes. After removing the flask from the water bath add 50 cc. distilled water and 2 cc. 10%  $\text{KI}$ . The iodine is determined in the colorimeter or by titration with thiosulfate. Estimate the amount of unconsumed  $\text{K}_2\text{Cr}_2\text{O}_7$ .

Jervell: *Acta. Med. Scand. Sup.* 24:19 (1928).

**Pyruvic Acid.** To 5 or 10 cc. biological fluid add a few drops of *o*-nitrobenzaldehyde, and then add 1 or 2 cc. of concentrated  $\text{KOH}$  and 10 cc. chloroform. Shake the solution, and if pyruvic acid is present a deep blue color will be formed.

Baeyer: *Ber.* 15:2856 (1882).

**Glyceric Aldehyde.** Two cubic centimeters of urine is distilled with a 12% solution of  $\text{HCl}$  in a micro-Kjeldahl apparatus and the total distillate made up to 25 cc., which is transferred to a 100-cc. flask. A drop of methyl orange is added, neutralized with 3 *N*  $\text{NaOH}$ , acidified with acetic acid, and 10 cc. of a standard water solution of phenylhydrazine is added. The liquid is kept in a water bath at  $50^\circ$  for 20 minutes to complete the precipitation of hydrazone. Cool, make volume up to 100 cc., and filter. The excess phenylhydrazine is determined in an aliquot portion of the filtrate. Into a flask is put 10 cc. of *N*/10 iodine solution and 10 cc. of the filtrate from the hydrazone. This is diluted to 100 cc. and the excess iodine determined in the colorimeter or by titration. The standard solution of phenylhydrazine is determined in a similar manner.

Ling and Nanji: *Biochem. J.* 15:466 (1921).

**Dihydroxyacetone.** Two cubic centimeters of protein-free tungstic acid blood filtrate is added to 2 cc. Folin-Wu phosphomolybdic acid

solution and heated in a test tube on a water bath for 15 minutes. After cooling, this blue solution is compared with a standard in a colorimeter.

Campbell: J. Biol. Chem. 67:59 (1926).

**d-Glucose.** (1) Dissolve 4 g. potassium ferrieyanide in a liter volumetric flask, dilute to volume. (2) Dissolve 16 g. anhydrous sodium carbonate in a liter volumetric flask with 80 cc. water, shake, and add 300 cc. freshly prepared 1% sodium cyanide solution, dilute to volume, and mix. (3) Dissolve 5 g. gum ghatti (India gum) in 800 cc. water, filter, and transfer to a liter flask. Add 5 g. anhydrous ferric sulfate in 76 cc. 85% phosphoric acid and 100 cc. water. Add 14 cc. 1% potassium permanganate solution to prevent the growth of bacteria. Dilute to volume and mix. (4) Mix 20 g. of neutral anhydrous  $\text{Na}_2\text{SO}_4$ ; 10 cc. of 10% sodium tungstate; 10 cc. of  $\frac{2}{3}N$   $\text{H}_2\text{SO}_4$ . Dilute to 250 cc. after dissolving. (5) Dissolve 99 mg. glucose in 100 cc. water. Add toluene and keep as stock solution (good 1 month) in refrigerator. For standard, dilute 1 cc. of stock solution to 100 cc. with tungstic acid solution. Call standard 0.01 mg. glucose per cc.

Blow 0.02 cc. blood into 2 cc. tungstic acid solution in a centrifuge tube, rinse pipet in solution and keep stirred 15 minutes, then centrifuge. Using dry pipet, transfer 1 cc. clear liquid to second tube graduated at 5 cc. Prepare standard in like manner and add to each 0.2 cc. ferrieyanide solution, 0.2 cc. carbonate cyanide solution; heat to boiling 8 minutes, cool under tap 1 minute, add 1 cc. iron ghatti solution, shake, and let stand 1 minute, fill to mark, and compare in colorimeter, using daylight passed through jar of potassium ferrieyanide solution, or place picric acid paper on mirror of colorimeter.

Folin and Malmros: J. Biol. Chem. 83:115 (1929).

Folin and Svedberg: J. Biol. Chem. 88:85 (1930).

McClendon: Proc. Soc. Exptl. Biol. Med. 27:773 (1930).

**Blood-Sugar** (R. B. Gibson). It is preferable that blood-sugars be taken by the finger-tip method and immediately placed in the tungstate.

However, blood taken by vein-puncture may be used, in which case the sample should be placed in tungstate solution within 15 minutes after withdrawing. Glycolysis takes place if the blood is allowed to stand, causing the blood-sugar values to be low. Normally, about 15 mg. of sugar disappears per hour, but this figure increases in diabetic bloods, according to Guest.

Glycolysis of a blood which stands for several hours, such as one

from out of town, may be avoided by using sodium fluoride as an anticoagulant.

Draw 0.2 cc. of blood from a finger-tip puncture (No. 11 Baird-Parker surgical knife blade) into a pipet, graduated to be used wet. Discharge the blood into a centrifuge tube containing 4.3 cc. of 1.25% sodium tungstate solution and 0.5 cc. of 2% sulfuric acid. (Spinal-fluid sugar determination: Use 0.4 cc. of spinal fluid, 4.1 cc. of tungstate, and 0.5 cc. of 2% sulfuric acid. The sugar value given in the table is divided by 2.) Centrifuge after standing 15 minutes; there will be sufficient fluid for duplicate determinations. Take 2 cc. of the supernatant fluid in a Folin-Wu sugar tube graduated at 10 and 25 cc. and 2 cc. of 0.01% glucose standard solution in a second tube; add 2 cc. of the alkaline copper tartrate solution to each tube, and heat for exactly 6 minutes in the boiling water bath. Cool and then add 2 cc. of sugar reagent to each tube and mix by inclining the tubes and tapping the bulbs of the tubes sharply against the palm of the hand. Dilute the blood-sugar tube to the 10-cc. mark and the standard tube to 25 cc. Mix and read in the colorimeter with the standard at 10 mm.; take the result from tabulated values for the ordinary range of readings. Normal fasting blood is about 90 mg. Blood specimens sent to the laboratory may be determined by the above procedure; 0.05 g. of sodium fluoride per 5 cc. of blood must be used as a preservative and anticoagulant. Non-sugar reducing substances in the blood do not affect the results obtained by this method.

For bloods with blood-sugar values over 300 mg., the blood tube should be diluted to the 25-cc. mark and the calculated result multiplied by 2.5. For hypoglycemic bloods a double strength alkaline copper solution is used; for 2 cc. of supernatant fluid, add 1 cc. of the double-strength copper solution and 1 cc. of a 0.004% glucose solution and subtract 50 mg. from the blood-sugar figure obtained.

*Solutions:* (1) Sodium tungstate, 1.25% solution.

(2) 2% sulfuric acid by volume (approximately  $\frac{2}{3}$  normal).

(3) Alkaline copper tartrate solution: Dissolve 16 g. of anhydrous sodium carbonate in 160 cc. of water in a 400-cc. beaker, add 3 g. of tartaric acid, and when dissolved add 1.8 g. of crystalline copper sulfate (grind in a mortar after weighing); mix and make up to 500 cc. (filter if necessary). A double-strength copper solution, twice the above ingredients made up to 500 cc., should be kept on hand.

(4) Standard sugar solution (stock): 1 g. of glucose to 100 cc.; add a few drops of xylene or toluene as a preservative and keep in the ice box. Dilute 1 cc. of the stock solution to 100 cc. for use to give a 0.01% solution. Dilute 1 cc. to 250 for 0.004.

(5) Sugar reagent: Arseno-phosphotungstic reagent as used in the uric acid determination plus 5 cc. of 40% formalin per 100 cc. Arseno-phosphotungstic acid reagent: 100 g. pure sodium tungstate in a liter flask plus 600 cc. water and dissolve. Add 50 g. of pure arsenic pentoxide plus 25 cc. of 85% phosphoric acid, plus 20 cc. of concentrated HCl. Boil 20 minutes. Cool and dilute to 1000 cc.

Gibson: Proc. Soc. Exp. Biol. Med. 27:480 (1930).

Blood sugar values from colorimetric readings (standard at 10 mm.) Micro-adaptation for finger-tip blood of the combined Folin-Wu and Benedict procedures; from sugar added to glycolized blood (Miss Baltimore):

mm.	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
2						400	385	372	362	349
3	337	325	316	306	297	289	281	273	266	259
4	253	247	241	236	231	226	221	217	212	208
5	204	200	196	192	189	186	182	179	176	173
6	170	167	165	162	159	157	155	152	150	147
7	145	142	140	138	135	133	131	129	126	124
8	122	120	118	116	114	112	110	109	107	105
9	104	102	100	99	98	96	95	94	92	91
10	90	89	87	86	85	84	82	81	80	79
11	78	77	75	74	73	72	71	69	68	67
12	66	65	64	63	62	61	60	59.5	59	58
13	57	56	55	54	53	52.5	52	51	50	49.5
14	49	48	47	46.5	46	45	44	43.5	43	42
15	42	41	40	39.5	39	38.5	38	37	36.5	36
16	35.5	35	34.5	34	33	32.5	32.5	32	31	31
17	30	29.5	29	29	28.5	28	27	27	26.5	26
18	25.5	25	24.5	24	24	23.5	23	23	22.5	22
19	22	21.5	21	20.5	20	20	19.5	19	19	18.5
20	18	18	17.5	17	17	17	16.5	16	16	15.5

**Urine Sugar.** Place 1 cc. urine in a test tube, add 9 cc. water from a Mohr pipet and about 1 g. HCl and HNO<sub>3</sub> washed Lloyd's reagent. Shake for 2 minutes; filter through a dry filter.

Pipet 0.5 cc. of this solution (or if strong test is obtained on qualitative, 0.2 cc.) into a Folin-Wu sugar tube, and 0.5 cc. of a 0.1% sugar standard to another tube. Add 4 cc. of Folin-Wu copper reagent to each tube. Heat in water bath for 6 minutes. Add 4 cc. of sugar reagent, agitate tubes, and dilute to 25 cc. Read in colorimeter with standard at 10. The values are read from a curve constructed from the following data. If 0.2 cc. is used multiply by 2.5.

Colorimeter reading

Standard at 10                      25.2   15.9   9.95   7.5   5.8   4.6

Per cent Sugar in Urine        0.25   0.5   1       1.5   2       2.5 (Cavett)

Gibson: Proc. Soc. Exp. Biol. Med. 27:480 (1930).

**Glucose in Urine** (Folin): (1) Dissolve 12 g. of sodium tartrate, 7 g. of anhydrous  $\text{Na}_2\text{CO}_3$ , and 20 g. of  $\text{NaHCO}_3$  in 700 cc. of water. Add 100 cc. of 5%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , dilute to 1 liter, and mix. (2) Dissolve 150 g. of sodium molybdate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ) in 300 cc. of water. Filter and wash filter paper with 75 cc. of water. Add several drops (0.1 to 0.2 cc.) of bromine and shake for a few minutes till the bromine has dissolved. Let stand for an hour to complete the oxidation produced by the hypobromite. Then add with shaking 225 cc. of 85% phosphoric acid. The surplus bromine is set free and imparts a yellow color to the solution. After all the phosphoric acid has been added, add also 150 cc. of cooled (25 volume %) sulfuric acid. Remove the surplus bromine by means of a moderately rapid air current. Add 75 cc. of 99% acetic acid, mix, and dilute to a volume of 1 liter. (3) Make a 1% stock solution of pure glucose in 0.25% benzoic acid. Transfer 1 cc. of stock solution by means of a pipet to a 100-cc. volumetric flask and 2 cc. to another; fill both to the mark with water to which has been added a few drops of toluene.

To 5 cc. of urine add 5 cc. of 0.05 *N* oxalic acid and 10 cc. water and mix. Add 1.5 g.  $\text{HCl-HNO}_3$ -washed Lloyd's reagent and shake gently for 4 minutes. Let the Lloyd's reagent settle as completely as possible; then filter the supernatant liquid into a small flask or beaker. Transfer 2 cc. of this filtrate to a blood-sugar tube graduated at 25 cc. and to two similar tubes add 2 cc. of standard sugar solution containing, respectively, 0.2 and 0.4 mg. copper solution. In each tube place 2 cc. of the copper tartrate solution. Transfer the tubes to a boiling water bath and heat for 10 minutes. Then transfer them to a cold water bath and let cool without shaking for 2 or 3 minutes. Add to each test tube 2 cc. of the molybdate phosphate solution. When the cuprous hydroxide is completely dissolved, dilute the resulting blue solutions to the 25-cc. mark, insert a rubber stopper, and mix by inverting each tube 3 times. The standard and the unknowns should be heated the same length of time, and should be at the same temperature when the reagent is added. Make the color comparison after 5 minutes and within 1 hour. Compare in a colorimeter with both standards, and if the results are not the same rely on the standard which most nearly matches the unknown.

Folin: *J. Biol. Chem.* 67:357 (1926); 70:405 (1926).

**d-Fructose.** One volume of the protein-free solution to be analyzed, 0.5 volume concentrated  $\text{HCl}$ , and 0.1 volume 20% alcoholic solution of diphenylamine are placed in a large test tube and heated in a boiling water bath for 15 minutes and cooled. The tube is kept closed with a 1-hole rubber stopper, the hole stuffed with glass-wool. If the experiment cannot be completed at once, stop at this point.

Shaking the solution with  $\frac{1}{2}$  volume of melted phenol causes the immediate absorption of the diphenylamine together with the color. The addition of 0.5 volume 95% ethanol renders the mixture homogeneous and suitable for colorimetric comparison. The color tends to darken slightly on standing. Standards are prepared similarly and simultaneously from a 1% stock solution of fructose preserved with toluene.

Corley: J. Biol. Chem. 81:81 (1929).

**Maltose.** To the unknown add 5 cc. yeast-water and sterilize in the autoclave for 10 minutes. Inoculate the mixture with a pure culture of *Saccharomyces exiguus* which will ferment glucose and sucrose but not maltose. Stopper the flask with a cotton plug and incubate at 25° for 3 weeks. Add 5 cc. alumina cream and boil the solution to expel the alcohol. Filter and wash the precipitate until the filtrate has a volume of 100 cc. On aliquots of the filtrate determine the reducing sugars, using glucose as a standard, and calculate the result of maltose.

Davis and Daish: J. Agr. Sci. 5:437 (1912).

**Lactose.** Dilute milk 1-100. Place 10 cc. diluted milk in centrifuge tube, add 0.5 g. solid picric acid and dissolve by stirring and shaking. Let stand for 10 minutes and centrifuge. Into a long test tube pipet 1 cc. of the supernatant liquid, and into a similar long test tube pipet 1 cc. of the standard (0.05 g. lactose in 100 cc. of saturated water solution of picric acid). Add 1 cc. saturated  $\text{Na}_2\text{CO}_3$  solution to both standard and unknown, mix, and immerse both tubes in boiling water for 20 minutes. Cool and dilute both solutions to 10 cc. and compare in colorimeter.

Pacini and Russel: J. Biol. Chem. 34:505 (1918).

**Glycogen.** An accurately weighed sample of tissue (5 to 15 mg.) is digested completely in a glass-stoppered test tube on a water bath by means of 0.1 cc. of 60% KOH for 1 to 2 hours, with occasional shaking. Glycogen is precipitated by the addition of 0.1 cc. of water followed by 0.6 cc. of 95% alcohol and 0.1 cc. of 1% aqueous  $\text{Na}_2\text{SO}_4$  solution, precipitated glycogen being carried down mechanically by the insoluble sulfate. The solution is allowed to stand for several hours, or better overnight, and the tube is then centrifuged. Supernatant liquid is removed by means of a special capillary pipet with the tip curved up. The precipitate is washed successively with 70%, 95%, and absolute alcohol, and then with petroleum ether, in portions of 0.3 cc., the precipitate being mixed and centrifuged each time. The remaining solvent is removed after the last washing by heating a short time on the water bath. The glycogen is hydrolyzed after

being mixed with 0.2 cc. of water and 0.3 cc. of normal HCl by being heated on a steam bath for 3 hours. After the hydrolysate is neutralized and diluted to a known volume, its glucose content can be determined by any standard method. Weight of glucose in the aliquot is multiplied by 0.93 to obtain the weight of glycogen to which it corresponds, since about 3% of the glycogen is lost during manipulations, and 10% of the glucose is water added by hydrolysis.

Osterberg: J. Biol. Chem. 85:97 (1929).

**Ellagic Acid.** Add a little  $\text{HNO}_3$  containing  $\text{HNO}_2$  which will give a blood-red coloration (Griessmayer's reaction).

Proctor-Paesser: Chem. Central-Blatt 1:235 (1901).

**Cysteine.** To 5 cc. standard cysteine solution (containing 0.05 mg. nitrogen per cc. 0.1 *N* HCl) and to 5 cc. of an unknown solution in 0.1 *N* HCl add 1 cc. 1% NaCN, mix, wait 5 or 10 minutes, add 1 cc. 0.5% water solution of 1.2 naphthoquinone-4-sodium sulfonate, and mix. Add 5 cc. 0.5 *N* NaOH. Let stand for a few minutes during which a red-brown color develops and then add 1 cc. 2% solution of  $\text{Na}_2\text{S}_2\text{O}_4$  causing the red-brown color to become a purer red.

Sullivan: U. S. Pub. Health Rep. 41:1030 (1926).

**Cystine.** In a 100-cc. Kjeldahl flask place 0.2 g. dried protein, add 5 cc. 6 *N*  $\text{H}_2\text{SO}_4$  and 1 cc. butyl alcohol; boil gently 18–20 hours on sand bath under a test tube condenser. Detach. Boil off butyl alcohol, add 1 g. kaolin, filter, wash, and dilute to mark in a 50-cc. volumetric flask and determine cystine by method given under nitrogen distribution.

Sullivan: U. S. Pub. Health Repts. 41:1030 (1926).

**Creatine.** Five grams of muscle is chopped fine and placed in a 200-cc. Erlenmeyer flask; 100 cc. 0.5 *N*  $\text{H}_2\text{SO}_4$  is added. The flask is covered with tinfoil and heated in an autoclave at 130° for about 45 minutes. After cooling below 100° the flask is removed; the contents cooled and transferred to a 200-cc. flask. This is diluted to the mark, mixed, and filtered, and the creatinine is determined.

Folin: J. Biol. Chem. 17:463 (1914).

**Creatinine.** By means of a pipet transfer 1 cc. urine into a 100-cc. volumetric flask. Into a similar flask transfer 1 cc. of a standard creatinine solution (1 g. creatinine in 1 liter of 0.1 *N* HCl). To each flask add 20 cc. saturated picric acid. (If determination cannot be finished, stop at this point.) Then add from a buret 1.5 cc. 2.5 *N* NaOH to each and let stand for 10 minutes; then dilute with water and mix well. Compare in colorimeter.

Greenwald: J. Biol. Chem. 14:87 (1913); 77:539 (1928).

**Purification of Picric Acid.** Four hundred grams of moist picric acid is placed in a 2-liter Pyrex flask, 1 liter pure benzene is added, and the crystals are dissolved by boiling. The solution is immediately poured through a fluted filter previously wet with benzene. Cover the filtrate with a watch glass and allow to stand overnight. The crystals adhere to bottom and sides of beaker. Pour out the benzene. Wash the crystals with benzene and dry in an air bath at 80°. (Explosive!)

**Phosphocreatine.** Cut and weigh muscle in a cold room, transfer to chilled mortar containing 20 or 30 cc. ice-cold 5% trichloroacetic acid (1:10). Let stand for 1 minute, pour off liquid, and grind muscle with twice its weight of cold quartz sand (5 minutes). Pour liquid back in and stir with pestle, repeat pouring, then filter mixture and make filtrate alkaline to phenolphthalein. Take 4 cc. of the neutralized trichloroacetic acid filtrate and pipet it into a centrifuge tube; treat with 1 cc. 10% solution of  $\text{CaCl}_2$  which has been saturated with  $\text{Ca}(\text{OH})_2$ . After 10 minutes the suspension is centrifuged for 2 minutes, the supernatant fluid is poured into a graduated test tube and the sediment washed with a mixture of 4 cc. of water and 1 cc. of the  $\text{CaCl}_2$  solution. The washings are added to the supernatant fluid. In a 50-cc. flask place 25 cc. water and 5 cc. 2.5% ammonium molybdate in 5 *N*  $\text{H}_2\text{SO}_4$ , and add the above solution. In  $\frac{1}{2}$  hour the color is developed by the addition of 2 cc. of  $\frac{1}{2}$ % (J.B.C., 66:388) aminonaphthol sulfonic acid solution and the usual standard prepared as nearly as possible at the same time. Inorganic phosphate may be determined by analyzing the calcium phosphate precipitate.

Fiske and Subbarow: J. Biol. Chem. 81:629 (1929).

**Tyrosine.** Transfer into a 100-cc. Kjeldahl flask 0.25 g. dried protein material. Add  $\frac{1}{2}$  cc. butyl alcohol (to prevent foaming), a couple of short spirals made from silver wire or silver foil (to prevent bumping), and 5 cc. 20%  $\text{NaOH}$ . Insert into the neck of the flask a condenser made from a test tube, and boil for 18 to 20 hours on sand bath. Remove the condenser, add 3 cc. water, and continue the boiling for 10 minutes to remove the alcohol. Remove the flame and from a pipet add immediately to the hot solution 2.5 cc. 14 *N*  $\text{H}_2\text{SO}_4$  (200 cc. concentrated  $\text{H}_2\text{SO}_4$  diluted to 500 cc.). Shake thoroughly and cool. Then add 1 cc. more of the 14 *N* acid, rinse the contents into a 25-cc. volumetric flask, dilute to volume, shake, and centrifuge in a closed tube. Transfer to a 15-cc. centrifuge tube 10 cc. of the unknown protein hydrolysate and add drop by drop from a height of about 3 cm. 4 cc. of a 15% solution of mercuric sulfate in 7 *N*  $\text{H}_2\text{SO}_4$ . No stirring is necessary. Let the mixture stand for 2 to 3 hours and centrifuge 5 minutes. Decant the supernatant liquid into a 100-cc.

volumetric flask draining thoroughly and rinsing the edge of the centrifuge tube with about 2 cc. of 0.1 *N* H<sub>2</sub>SO<sub>4</sub>. To the sediment in the tube add 10 cc. of a solution containing 1.5% mercuric sulfate in 2*N* sulfuric acid. Stir with a fine glass rod and let stand for 10 minutes. At the end of 10 minutes rinse the stirring rod into the centrifuge tube with 2 cc. of the same 1.5% mercuric sulfate solution. Centrifuge again and transfer this wash liquid to the flask containing the original mother liquor, rinsing the edge of the centrifuge tube. Introduce into a second 100-cc. volumetric flask 5 cc. of a standard tyrosine solution in 2*N* H<sub>2</sub>SO<sub>4</sub> containing 1 mg. tyrosine per cc. Add 4 cc. of the 15% mercuric sulfate solution and 12 cc. of the 1.5% mercuric sulfate solution and 7 cc. of 0.1 *N* H<sub>2</sub>SO<sub>4</sub>. To the standard and the unknown add 6 cc. of 7*N* H<sub>2</sub>SO<sub>4</sub>. Heat the two flasks in boiling water for 15 minutes and then cool in cold water approximately to room temperature. Add to each flask with shaking 1 cc. 2% sodium nitrite solution. Dilute to volume at once and make the color comparison without undue delay.

Folin and Ciocalteu: *J. Biol. Chem.* 73:627 (1927).

**Tryptophan.** Five cubic centimeters of unknown solution is treated with 5 cc. 15% HCl (containing 12 cc. 0.1% formaldehyde per liter); 10 cc. concentrated H<sub>2</sub>SO<sub>4</sub> is added and the mixture carefully shaken until no more bubbles appear. If tryptophan is present a blue-violet color appears and does not fade for 24 hours. The color is compared in a colorimeter with that of a standard treated similarly.

Komm and Bohringer: *Z. physiol. Chem.* 124:287 (1923).

**Van Slyke-Cavett Nitrogen Distribution Method.** One-half gram of the protein is hydrolyzed by boiling for 36 hours with 7 cc. of 25% hydrochloric acid in a 250-cc. distilling flask. A test-tube condenser is inserted in the neck of the flask. After the digestion the flask containing the protein hydrolysate is placed in a water bath at 60° and vacuum-distilled until a paste remains. A few cubic centimeters of water are added and the process repeated.

**Ammonia Nitrogen** (fig. 57). Place 10 cc. of 0.1 *N* acid, 100 cc. water, and 3 drops of methyl red in a 500-cc. suction flask. The distillation flask containing the hydrolysate setting at a 45° angle is connected to a small Hopkins condenser which extends to the bottom of the suction flask. Thirty cubic centimeters of water, 1 cc. of butyl alcohol, and 0.5 g. of calcium oxide are added to the hydrolysate and the flask closed with a stopper carrying a capillary tube to the bottom of flask. It is vacuum-distilled at 45–50° until 10 cc. remain.

**Humin Nitrogen.** The contents of the distillation flask, from which the ammonia has been removed, are transferred to a 50-cc. centrifuge

tube. The flask is rinsed with small portions of water, as the total volume *must not* exceed 25 cc. The humin material is separated by centrifugation and washed 3 times with 5-cc. portions of water by centrifugation.

The washed precipitate is subjected to Kjeldahl digestion as for total nitrogen, except that it is carried out in the distillation flask which still contains some adhering humin.

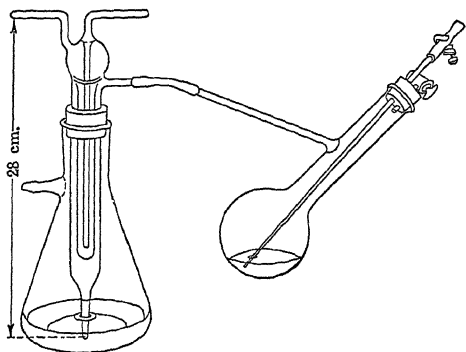


FIG. 57. Ammonia nitrogen distillation apparatus. J. Biol. Chem.

**Phosphotungstate Precipitation and Separation.** The mother liquor and washings from the humin precipitate are collected in a 100-cc. centrifuge tube. Five cubic centimeters of concentrated HCl are added, followed *immediately* by 2.5 g. of phospho-24-tungstic acid dissolved in a few cubic centimeters of hot water. The volume is adjusted to 50 cc. The tube is placed in a hot water bath for 1 hour. It is allowed to cool, stoppered, and placed in an ice box at 0° for 48 to 72 hours. Several hours before the final separation is made the tube is shaken in such a manner as to cause floating particles and those adhering to the side to sink. It is then centrifuged and returned to the ice box.

For the final separation and washing of the phosphotungstate precipitate the tube is again centrifuged, care being taken to see that floating or adhering particles sink to the bottom. The liquid is gently decanted into a 100-cc. volumetric flask and the centrifuge tube chilled in ice water. The precipitate is washed three times with 4-cc. portions of cold acid mixture (10 cc. of concentrated hydrochloric acid and 2.5 g. of phosphotungstic acid per 100 cc. of solution). Each time the precipitate is broken up with a stirring rod. After centrifugation the wash liquid is added to the original solution in the 100-cc. flask. The solution is then neutralized by adding 1:1 sodium hy-

dioxide solution until a white precipitate begins to form. This is *immediately* dissolved with glacial acetic acid. After room temperature is reached, the solution is diluted to volume.

The phosphotungstate precipitate remaining in the centrifuge tube is dissolved by suspending it in 5 cc. of water and adding 4 or 5 cc. of *N* sodium hydroxide. Any undissolved material is centrifuged out and the clear liquid is decanted into a 50-cc. volumetric flask. A few cubic centimeters more of water and *N* sodium hydroxide are again added to the undissolved precipitate remaining in the centrifuge tube and the above process repeated. Sometimes a third addition of water and sodium hydroxide is required for complete solution. The solution is neutralized and diluted to volume.

**Analysis of Phosphotungstate Precipitate and Filtrate. Nitrogen.**

Nitrogen is determined in duplicate upon 5-cc. portions of the basic fraction and 25 cc. of the filtrate. For the digestion of the filtrate fraction 6 cc. of the concentrated sulfuric acid is used instead of 4 cc.

*Amino Nitrogen.* This is determined on 2-cc. portions of the basic and the filtrate fractions with Van Slyke's micro amino nitrogen apparatus.

*Arginine Nitrogen.* Ten cubic centimeters of the basic fraction solution are placed in a 300-cc. Florence flask, 10 cc. of 1:1 sodium hydroxide solution is added and connected to the arginine apparatus (fig. 58). The large test tube contains 10 cc. of 0.02 *N* acid. Water is allowed to pass through the condenser and the contents of the flask are boiled gently over a micro-burner for 6 hours. The apparatus is allowed to cool and the condenser is drained. Ice and water in a beaker are placed around the test tube which contains the standard acid. The last traces of the ammonia are then steam distilled into the acid. The titration is made with 0.02 *N* alkali with the micro-buret.

*Cystine Nitrogen.* (Folin and Marenzi.) Five cubic centimeters of the solution obtained from the phosphotungstate precipitate are placed in a 25-cc. volumetric flask and 2 drops of 50% sulfuric acid added, thus producing an acidity similar to that of the standard solution. The standard cystine solution is a solution of *N* sulfuric acid containing 17.16 mg. of cystine per 100 cc.; thus 1 cc. contains 0.02 mg. of cystine

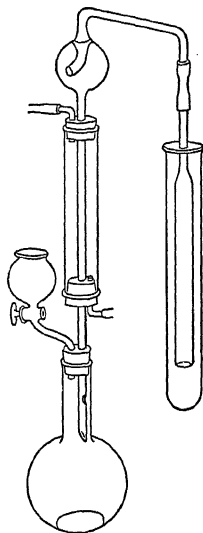


FIG. 58. Arginine nitrogen apparatus. For steam distillation replace the separatory funnel with a tube leading to the bottom of the flask. J. Biol. Chem.

nitrogen. The necessary amount of the standard solution (usually 2 to 5 cc.) is added to a 25-cc. volumetric flask. The volume of liquid in the standard flask, during the color development, should be equal to that in the unknown flasks. One-half cubic centimeter of freshly prepared 20% sodium sulfite (Merck) solution is added to the standard and unknown. After this has stood for 1 minute, 4.5 cc. of a 20% solution of sodium carbonate, 0.5 cc. of 20% lithium sulfate, and 2 cc. of Folin's new molybdate-free uric acid reagent are added. After standing for 3 to 4 minutes, the solutions are diluted to volume with a freshly prepared 3% solution of sodium sulfite and compared in a colorimeter.

The cystine which is not precipitated and remains in the filtrate may also be determined by placing 10 cc. of the filtrate solution in a 50-cc. centrifuge tube graduated at 25 cc. The reagents are added as for the basic fraction, except that the solution is immediately diluted to 25 cc. and centrifuged before comparison with the standard.

*Histidine.* (Koessler and Hanke.) An aliquot of the basic fraction is diluted 1:10 and 1 cc. of this solution is used for the determination.

One and one-half cubic centimeters of sulfanilic acid solution (0.9 g. of sulfanilic acid dissolved in 9 cc. of 37% hydrochloric acid and diluted to 100 cc.) is placed in a 50-cc. flask immersed in ice-water and 1.5 cc. of 5% sodium nitrite solution added. After 5 minutes 6 cc. more of the nitrite solution is added. In 5 minutes the solution is diluted to 50 cc., and is ready for use after 15 minutes.

Eight cubic centimeters of 1.03% pure anhydrous sodium carbonate solution is placed in a large test tube; 3 cc. of the above reagent (*p*-diazobenzene sulfonic acid) is added, the tube shaken, and in exactly 1 minute 1 cc. of the unknown solution is added and thoroughly mixed. After 6 minutes the solution is compared in a colorimeter with an artificial standard.

The artificial solution is prepared by diluting 0.82 cc. of 0.5% Congo red solution with 300 cc. of water. To this is added 0.9 cc. of 0.1% methyl orange solution, and the volume is brought to 500 cc. This artificial standard should give a color equivalent to 0.009 mg. of histidine nitrogen in 12 cc. but should be rechecked against a standard histidine solution whenever a new set of reagents is used, owing to variations in the purity of the reagents and hence the color produced. A convenient stock standard for this purpose contains 5.43 mg. of histidine dichloride (1 mg. of nitrogen) in 1 cc. of 0.01 *N* hydrochloric acid. One cubic centimeter of the stock standard and 0.5 g. of phosphotungstic acid diluted to 100 cc. serve as a working standard and contain 0.01 mg. of histidine nitrogen per cc.

Sometimes a perfect color match is not obtained, but no difficulty is

experienced in reading if Bausch and Lomb blue filter No. 3610 is used since the color absorption of this solution is in the short wavelengths.

*Nitrogen Distribution References:*

Cavett: J. Lab. and Clin. Med., 17:79 (1931).

— J. Biol. Chem., 95:335 (1932).

Holm: J. Am. Chem. Soc., 42, 611 (1920).

Folin and Marenzi: J. Biol. Chem., 83, 103, 109 (1929).

Koessler and Hanke: J. Biol. Chem., 39, 497 (1919).

Hanke and Koessler: J. Biol. Chem., 43, 527 (1920).

Van Slyke: J. Biol. Chem., 10:15 (1911).

**Reduced Glutathione.** Grind 2 g. tissue in a mortar with 3 g. clean sand and 2 cc. 10% trichloroacetic acid. Centrifuge the resulting mass and extract twice with 2-cc. portions of 10% trichloroacetic acid. Place the combined filtrates into a 20-cc. separatory funnel; add 2 cc. 25% KI. Add  $N/100 I_2$  until a color appears, and determine excess iodine by 2 extractions with 1 cc.  $CCl_4$  each (p. 306). One cubic centimeter  $N/100 I_2 = 2.5$  mg. reduced glutathione.

Perlzweig and Delrue: Biochem. J. 21:1416 (1927).

**Biuret** is determined by adding strong alkali and a trace of  $CuSO_4$  to unknown and standard and comparing in colorimeter. The standard is made as follows: Heat gradually to  $140^\circ$ , 50 g. urea in a 500-cc. balloon flask, introducing at the same time dry HCl until the first resulting oily liquid becomes firm. Extract the reaction product with a little water. Suspend the residue in 10 volumes boiling alcohol. For each 10-g. reaction product add 6 g.  $Ca(OH)_2$  suspended in boiling alcohol. Boil in a reflux condenser for  $\frac{1}{2}$  hour, filter off the undissolved calcium cyanurate, and neutralize the filtrate with HCl, keeping the solution hot. Cool and filter off the precipitated biuret.

Beilstein: Handbuch der organischen Chemie 4, (3):70 (1921).

**Ammonia.** The ammonia in the urine is adsorbed on permutite; the permutite is removed; the ammonia is then released by NaOH and determined by Nesslerization. Place 1 g. permutite in a 100-cc. volumetric flask; add 3 cc. HOH and 1 cc. urine. Rinse down with 1-3 cc. HOH and shake gently for 5 minutes. Rinse the permutite to the bottom with 15-20 cc. water ( $NH_3$ -free), and decant. Repeat twice. Add a little water, 1 cc. 10% NaOH, shake 20 seconds, and dilute to 75 cc.

To prepare the standard solution add 5 cc.  $(NH_4)_2SO_4$  (containing 0.1 mg. nitrogen per cc.) to 1 cc. 10% NaOH; mix, and dilute to 75 cc. Add 5 cc. Nessler's solution (see total nitrogen) to the standard with shaking. The resulting solution must be clear; if not, prepare again.

Then in the same manner add 5 cc. Nessler's reagent to the unknown. Dilute each flask to 100 cc. and compare in a colorimeter.

Folin and Bell: *J. Biol. Chem.* 29:329 (1917).

**Methylamine.** The water solution to be tested is treated with 1 volume of benzene-sulfon-chloride which has no action on tertiary amines in alkaline solution. KOH is added until the chloride odor disappears. It is then extracted with 2(50-cc.) portions of ether to remove secondary and tertiary amines. The residual water solution, after ethereal extraction, is treated with an excess of NaOH and distilled from a volume of at least 80 cc. The first 10 cc. which is distilled over is caught in 10 cc. 0.5% alcohol solution of 2-4 dinitrochlorobenzene and allowed to stand for about 20 hours. The 2-4 dinitromethylaniline is then recrystallized from ethyl alcohol, dissolved in ether or other solvent and compared with a standard in a colorimeter, and the amino nitrogen is calculated.

Hinsberg: *Ber.* 23:2962 (1890).

Sidgwick: *Organic Chemistry of Nitrogen*, 1:19, Oxford (1910).

**Adrenaline.** Pipet 5 cc. of the clear unknown solution into a 100-cc. volumetric flask. Into a similar flask pipet 1 cc. of a fresh uric acid solution (0.02% uric acid in water containing 0.1%  $\text{NaH}_2\text{PO}_4$ , 0.9%  $\text{Na}_2\text{HPO}_4$ , 0.14% acetic acid). To each flask add 2 cc. uric acid reagent (p. 350) and 20 cc. saturated sodium carbonate solution. After allowing to stand for 2 or 3 minutes dilute to the 100-cc. marks, shake thoroughly, and compare in colorimeter. Epinephrine gives 3 times as much color as uric acid per unit weight.

Folin, Cannon, and Denis: *J. Biol. Chem.* 13:477 (1912).

**Indole.** Place 5 g. feces in an 100-cc. Kjeldahl flask and add 70 cc. slightly alkaline water. Simultaneously place 1 cc. indole solution containing 0.01 mg. in a similar flask and treat similarly throughout the determination. The flasks are set up for distillation with steam. One cubic centimeter 10% KOH and 3 drops paraffin oil are added. Distil 50 cc. Take 10-cc. aliquots of distillates and transfer them to 50-cc. Erlenmeyer flasks. Add to each, 2 g. washed permutite. Rotate the flasks and contents moderately for 10 minutes; then as soon as the permutite settles, pour the liquids into 25-cc. separatory funnels. Add 0.1 cc. of a 2% solution sodium  $\beta$ -naphthoquinone-4-sulfonate and 0.2 cc. 10% KOH, and allow to stand for 15 minutes. A greenish blue color develops and is extracted twice with 1 cc.  $\text{CCl}_4$ , and compared in a colorimeter.

Bergeim: *J. Biol. Chem.* 32:17 (1917).

**Indoxyl Sulfuric Acid.** Place 10 cc. protein-free urine in a test tube. Add an equal bulk of concentrated HCl containing 0.4%

ferric chloride and mix. Allow to stand for 10 minutes and then add 3-5 cc.  $\text{CCl}_4$ . Mix by pouring from one test tube to another and allow to stand for 15-30 minutes, and separate in separatory funnel. Compare the  $\text{CCl}_4$  solution in a colorimeter with a solution containing 0.009 mg. pure indigo per cc.  $\text{CCl}_4$ . (Color due to presence of thymol or of KI disappears on addition of NaOH or of sodium thiosulfate.)

Rosenbloom: N. Y. Med. J. 98:314 (1913).

**Urea in Blood.** Prepare a protein-free filtrate from 0.2 cc. of blood according to the Gibson method for blood-sugar. Transfer 4 cc. to a large Pyrex test tube. Add 0.1 cc. acetate buffer (15 g. crystalline sodium acetate and 1 cc. glacial acetic acid per 100 cc.), and 1 cc. of urease solution (place in flask 1 g. of jack bean meal. Add 40 cc. of 30% alcohol. Shake 10 minutes and centrifuge or filter. Place in filtrate 1 g. of permutite that has been washed once with 2% acetic acid and twice with water, and store in refrigerator).

NOTE: Urease is destroyed by mercury. If apparatus with which it comes in contact has contained mercury solutions, the apparatus should be washed with nitric acid and rinsed well before use.

Stopper and incubate 10 minutes at  $45^\circ$  or 25 minutes at room temperature. Place in test tube a very small U-tube with open ends down, 1 drop of lubricating oil, and finally 2 cc. of saturated borax solution. Connect at once with distilling tube and distil as in the determination of nitrogen. The receiver should contain 1 cc. of 0.1 N HCl. After about  $\frac{1}{3}$  of the solution has distilled over, lower the receiver so that the inside of the delivery tube is rinsed out. Rinse the outside. Dilute to 10 cc. Prepare standard (see nitrogen determination) of  $(\text{NH}_4)_2\text{SO}_4$  to contain in 10 cc. 0.04 mg. nitrogen. Nesslerize both solutions with 1 cc. Nessler's solution (see nitrogen determination), and compare in colorimeter.

For urea in urine, dilute urine 1:1000 and run determination as above on 4 cc. of the diluted solution. For accurate work it is desirable to run blank determinations on the reagents.

**Uric Acid.** Transfer 1 cc. urine to a centrifuge tube and add water to 6 cc. Add 5 cc. silver lactate solution. (Dissolve 40 g. silver nitrate in 1 liter of water and add slowly while stirring 60 cc. 20% sodium carbonate. Test supernatant fluid with sodium carbonate, and if a precipitate appears add more sodium carbonate. When silver is completely precipitated, decant and filter and wash precipitate several times. Dissolve precipitate in 1 liter 7.5% lactic acid solution and keep in dark.) Stir with a glass rod. Rinse off rod with a few drops water. Centrifuge for 2-3 minutes. Add a drop of silver lactate so as to be sure that an excess is present; if a precipitate ap-

pears add 2 cc. more and centrifuge again; if no precipitate appears pour off liquid as completely as possible. To the precipitate in the centrifuge tube add 4 cc. 5% sodium cyanide solution. Stir until clear. Rinse stirring rod, collecting the rinsings in a 100-cc. graduated flask and rinse the tube 3 times with 5 cc. water. Fill to 100-cc. mark with water and mix. Analyze as follows:

*Uric Acid in Protein-free Blood Filtrate or Urine Centrifugate.* Pipet 5 cc. into a test tube and add 5 cc. water. Place in a similar tube 5 cc. diluted standard uric acid solution containing 0.02 mg. uric acid. (Dissolve 9 g.  $\text{Na}_2\text{HPO}_4$  and 1 g.  $\text{NaH}_2\text{PO}_4$  in 300 cc. hot water. Filter. Make up to 500 cc. with hot water and pour on 200 mg. uric acid suspended in a few cubic centimeters of water in a liter volumetric flask. Add 1.4 cc. acetic acid. Dilute to volume. Preserve with  $\text{CHCl}_3$ . Dilute 50 times before use.) Add 5 cc. water. To both standard and unknown add 4 cc. 5% sodium cyanide and immediately 1 cc. arsenic phosphotungstic acid reagent. (Place 100 g. sodium tungstate in 600 cc. water in 1-liter flask. Add 50 g. arsenic pentoxide, 25 cc. 85% phosphoric acid and 20 cc. concentrated  $\text{HCl}$ ; boil 20 minutes, cool, and dilute to 1 liter.) Mix by inverting once, and place in a boiling water bath for 3 minutes. Read in colorimeter within 5 minutes, setting unknown at 20 mm.

Benedict: J. Biol. Chem. 51:187 (1922).

Folin and Denis: J. Biol. Chem. 38:459 (1919).

**Chlorophyll** is determined directly; the standard is made as follows: Dry leaves away from sunlight until they crumble in the hands. Grind and place 1 kg. in a 25-cm. Büchner funnel with 2 filter papers and pour 2 liters of 80% (by volume) acetone over it. Let run through, then apply suction. Extract acetone solution with 1 liter petroleum ether in separatory funnel. Run acetone layer into another separatory funnel containing 1 liter petroleum ether. Finally pour acetone layer into separatory funnel containing 0.5 liter petroleum ether. Wash petroleum ether in 3 separatory funnels with 1 liter 80% acetone. Allow acetone to run in fine streams through petroleum ether solutions. Remove acetone by running 1 liter distilled water through the separatory funnels in succession. Allow 4 liters 80% methyl alcohol (by volume) to run down in a fine stream through petroleum ether in separatory funnels. Allow the methyl alcohol extracts to run out of each separatory funnel into the next as rapidly as it separates from the petroleum ether layers. Remove methyl alcohol and acetone by allowing a fine stream of water to run through the petroleum ether solutions. Shake petroleum ether solution with 250 g. anhydrous sodium sulfate. Filter through 3 layers of talc on 25-cm. Büchner funnel. Break layer of chlorophyll

on talc with nickel spatula to increase filtering efficiency. Use moderate suction. Wash chlorophyll with 500 cc. petroleum ether. Apply strong suction to remove petroleum ether. Remove chlorophyll talc to beaker and stir for a short time with 500 cc. acetone. Filter solution in Büchner funnel (15 cm.) and wash talc with acetone till all chlorophyll is removed. Pour acetone solution into a separatory funnel containing 1 liter petroleum ether and add 500 cc. to each of 2 other separatory funnels. Add water to acetone solution. Run off water layer into petroleum ether to recover acetone. Wash petroleum ether with 8-12 liters of water to remove acetone. Dry petroleum ether with sodium sulfate. Filter in 15-cm. Büchner funnel. Wash with 1 liter petroleum ether. Allow to stand to remove the ether. Purify by dissolving in alcohol-free ethyl ether and filtering over talc. Wash with 1 liter petroleum ether. Wash with ether and evaporate to syrupy mass. Use reduced pressure near close of evaporation. Precipitate by shaking with petroleum ether. Filter. Repeat extraction and concentrate and dry in beaker in vacuum desiccator. Dissolve in acetone and compare in a colorimeter.

Schertz: *Plant Physiol.* 3:487 (1928).

**Hemoglobin.** Place Bausch and Lomb blue filter, No. 3610, on eyepiece of colorimeter. Place yellow filter No. 3611 on left side. Draw blood into the special pipet to the 10 mark and dilute to 5020 mark with 0.1 *N* HCl, noting time of dilution. Empty pipet into right cup. Fill left cup with distilled water. Secure match by adjusting height of acid hematin, readjusting the distilled water height to approximately the same value. Read grams hemoglobin per 100 cc. from Bausch and Lomb chart, correcting for time elapsing between dilution and reading by reference to time-correction table.

Newcomer: *J. Biol. Chem.* 55:569 (1923).

**Hematin** is determined by the method for hemoglobin after adding gelatin to the hematin to keep it in solution. A standard hematin solution may be prepared (by the method of Elvehjem or) as follows, although such standards are not always satisfactory: Dilute washed blood corpuscles with water and carefully add acid. Coagulate by heating. Filter with suction and press as dry as possible. Rub the mass with 95% alcohol previously treated with oxalic acid or  $\frac{1}{4}$ -1%  $\text{H}_2\text{SO}_4$ . Allow to stand at room temperature for several hours. Warm the filtrate to 70° and treat with alcoholic HCl (2.5 cc. Conc. HCl + 7.5 cc. 95% alcohol for each liter of filtrate). Allow to cool. Let stand from 1 to 2 days until crystals form. Dissolve

crystals in very dilute caustic alkali in presence of oxygen. Hematin crystals form upon standing.

Elvehjem: J. Biol. Chem. 93:203 (1931).

Schumm and Mertens: Z. physiol. Chem. 168:1 (1927).

**Bilirubin** is determined directly. The standard is made as follows: Heat bile with 100 cc. methyl alcohol while passing in  $\text{NH}_3$ . Shake the mixture for 1 hour and again heat and treat with  $\text{NH}_3$ . Filter off the insoluble material and wash residue with 10 cc. methyl alcohol saturated with  $\text{NH}_3$ . Pour the filtrate and washings into 1 liter of ether and place in freezing mixture where bilirubin ammonia precipitates as microscopic crystals. It decomposes into bilirubin by boiling with methyl alcohol.

Kuster: Z. physiol. Chem. :279 (1924).

**Urobilinogen.** One cubic centimeter of Ehrlich's reagent is added to 10 cc. whole urine and compared with a standard in a colorimeter.

Diamond and Wallace: Arch. Internal Med. 35:698 (1925).

**Urochrome** is determined directly. The standard is made as follows: Evaporate fresh urine on a water bath, treat with cold  $\text{HCl}$  and extract with ether. Distil the ether off and mix the residue with water. Hippuric acid is deposited: filter and wash with water. A deposit of resin is now produced. Separate after settling by filtration. To the filtrate a solution of basic lead acetate is added until no further precipitation takes place. The precipitate is collected on a filter and washed with hot water. The salt is decomposed by cautiously adding a slight excess of dilute  $\text{H}_2\text{SO}_4$  while triturating in a mortar — immediately neutralized by adding  $\text{BaCO}_3$ . Filter and add  $\text{Ba(OH)}_2$  until alkaline. Neutralize with  $\text{H}_2\text{CO}_3$  and allow to stand. To this, pure neutral mercuric acetate is added until no more precipitate is formed. Filter, wash, again triturate, and wash on filter. If the precipitate is gray or dark colored, treat with  $\text{H}_2\text{S}$  in water and again with  $\text{Ba(OH)}_2$  and mercuric acetate. If bay-colored, collect in a flask of water and pass a current of  $\text{H}_2\text{S}$  through, boil in current of hydrogen, cool and filter

Thudicum: Brit. Med. J. 2:583 (1864).

**Uroerythrin** is determined directly. The standard may be made as follows: Take a healthy one-day-old 24-hour sample of amber-colored urine and shake with 10–15 g. talc, let stand several hours, and decant. Collect the precipitate and wash with the aid of a suction pump. Mix the colored talc paste with 95% alcohol containing 0.5%  $\text{HCl}$ , warm in a test tube to 40–50°, and filter. The sediment

contains much uroerythrin attached to uric acid and urates. Mix it with an equal amount of talc and 150-200 cc. water and extract the uroerythrin as before. Uroerythrin is easily soluble in alcohol acidified with HCl, and the solution is orange colored.

Borrien: J. pharm. Chim. 16:45 (1917).

# PART VI.—TABLES

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Acetaldehyde.....	44.00	-133.5	30.3	0.783 (20)	liq. col.	misc.	misc.	misc.	an intermediate in carbohydrate and fat metabolism and alcoholic fermentation.
Acetamide.....	135.08	114.2	369.9	1.211 (4)	wh. leaf.	0.5	40	8.3	an analgesic and antipyretic.
Acetarsone.....	275	265	.....	.....	monoclin.	i.	s.	s.	an antiparasitic arsenical.
Acetone.....	58.08	-94.3	56.1	0.792 (20)	liq. col.	misc.	misc.	misc.	is formed by decarboxylation of acetoacetic acid in diabetes.
Acetophenetidine.....	179.11	134.7	d.	.....	leaf.	0.11	6.0	1.3	an analgesic and antipyretic.
Acetylcholine.....	183.2	.....	.....	.....	.....	.....	.....	.....	an alkaloid found in ergot; it lowers blood pressure and increases intestinal movement, a hormone.
Acetylene.....	26.02	-81.8	-83.5	0.813 - 80	gas	v.s.s.	s.	25/1 aet.	an anesthetic when mixed with 2 volumes of air and inhaled.
Acetyl salicylate.....	180.09	133.5	.....	.....	column. cryst.	s.s.	s.	s.	is an analgesic and antipyretic.
Acid, acetic.....	60.05	16.7	118.1	1.051 (20)	liq.	misc.	misc.	misc.	is an intermediate in metabolism; the laxative action of roughage is due to acetic s. fermentation.
Acetoacetic.....	102.05	32.0	85.0 (21)	1.200 (17)	oil	misc.	misc.	misc.	is derived from $\beta$ -hydroxybutyric acid in the body; it is increased in diabetes.
Acetoin.....	174.05	161.2	.....	.....	leaf.	18	50 (12)	s.s.	occurs in plasma.
Acrylon.....	73.08	12.3	141.9	1.082 (14)	liq.	misc.	.....	.....	has a pungent odor.
Adanylin.....	347.13	197	.....	.....	need.	.....	.....	.....	a nucleotide from nucleic acid from cell nuclei which lowers blood pressure and intestinal motion.
Adipin.....	146.08	151	246 (100)	.....	need.	1.5 (15) v.s.	.....	v.s.	is formed by oxidation of fat.
Aldehydic from Type.....	356	.....	.....	.....	.....	.....	.....	.....	glucose $\beta$ -glyceronic acid.
A Friedlander B.....	.....	.....	.....	.....	.....	.....	.....	.....	isomeric with the above; a hydrolysis product of the antigen.
Aldehydic from Type.....	356	.....	.....	.....	.....	.....	.....	.....	.....
III Paeumococcus.....	350.02	.....	.....	.....	.....	.....	.....	.....	.....
$\alpha$ -Amino butyric.....	103.08	d.385	.....	.....	leaf.	1/3.5	1/300h	.....	a synthetic amino acid.
Arginin.....	106.09	45.0	185	0.868 (47)	monoclin.	s.s.	v.s.h.	v.s.	occurs in Argon.
Arsetidic.....	312.23	77	238	.....	lvs.	i.	0.45 (20)	v.s.	occurs in fats.
Arsethodonic.....	304.26	.....	245	.....	liq.	.....	.....	.....	occurs in oils.
Aspartin.....	133.06	270	.....	1.621 (4)	leaf.	0.80 (20)	labe.	i.	an amino acid found in proteins.
Atropin.....	146.96	107	297 d.	.....	monoclin. tab.	0.14 (19)	s.	St. (C <sub>2</sub> )	is derived from atropine.
Adalin.....	138.13	106.5	396 d.	1.029	leaf.	0.34 (20)	v.s.	2.7	is derived from oleic acid.
Behestin.....	340.35	94	394 (80)	.....	colic. tab. need.	i.	0.10 (17)	1.02 (16)	occurs in behest oil.
Beronic.....	112.05	121.7	349.2	1.266 (13)	col. leaf.	5.0 (20)	47 (15)	31.4	occurs in benzoin and cranberries, 0.1% is antiseptic, 10 g. in 4 hours is toxic to man.
.....	.....	.....	.....	.....	or need.	0.29 (20)	.....	.....	.....
Boric.....	61.84	185 d.	300	1.424 (15)	triad. wh.	5.15 (21)	s.	0.34 (25)	2.5% solution is antiseptic; used as a pH buffer.
$\alpha$ -Butyric.....	88.08	-9.9	183.0	0.864 (20)	liq.	misc.	misc.	misc.	is a constituent of butter; the laxative action of roughage is partly due to it produced by fermentation.
Caodylic.....	138.02	360 d.	.....	.....	rhomb.	v.s.	v.s.	v.s.s.	an arsenical antiparasitic.
Calicin.....	130.06	195	d.	.....	yel. monoclin.	s.	v.s.	.....	occurs in black fir resin.
Cagrin.....	172.16	31.0	268.4	0.93 (27)	col. need.	v.s.s.	s.	s.	is a constituent of butter and goat fat.
Cagrin.....	118.1	-9.5	202.0	0.826 (21)	liq.	v.s.s.	s.	s.	is a constituent of butter and goat fat.
Cagrin.....	144.13	16.0	237.5	0.91 (20)	col. leaf.	0.35 (20)	misc.	misc.	is a constituent of butter and goat fat.
Carbamin.....	90.02	.....	.....	.....	.....	s.	.....	.....	an amino acid not known in the free state; a hypothetical intermediate in urea formation.
Carminin.....	404.18	156 d.	.....	.....	red powd.	v.s.	s.	v.s.s.	a glycoside in cochineal "insect" used as a biological stain.
Carminic.....	368.37	72	.....	.....	.....	.....	.....	.....	occurs in beef kidney and carminic wax.
Casacillin.....	184.15	-15	270	0.863 (20)	.....	.....	.....	.....	occurs in casacillin oil.
Casotic.....	308.42	82.5	d.	0.838 (73)	need. f.s.	i.	v.s.s.	20 (35)	occurs in wax.
Chaulimogrin.....	290.25	69	345 (20)	.....	.....	.....	s.	.....	occurs in chaulimogrin oil; 1/100,000 is antiseptic to leprosy germs.
Cholein.....	.....	190	.....	.....	lvs. pr.	1/2000	i.	al. s.	is formed by combination of dihydroxycholesterol s. and fatty a.; occurs in bile.
Cholestin.....	406.34	165	.....	.....	rhomb. pl.	v.s.	s.	.....	occurs in bile, reduces surface tension of water.
Chlodivitin sulfuric.....	.....	.....	.....	.....	amorph. p.	s.	.....	.....	a hydrolytic product of cartilage.
Chrysophanic.....	254.08	191	subl.	.....	or. need.	i.	0.445	s.	a purgative dye from cassia also used in treating pericarditis.
Cinamin.....	246.22	44.2	.....	.....	prin.	.....	.....	.....	occurs in bedbugs.
Cinazolin.....	146.06	133	300	1.284 (4)	col. monoclin.	0.1 (20)	20 (20)	v.s.	occurs in balsams, is antiseptic.
Citrin.....	132.06	153	d.	1.542 (18)	col. rhomb.	138c.	119 (24)	2.26	occurs in plants and animals; lessens the ionization of Ca and prevents blood coagulation.
Citronellin.....	275	50	222	.....	oil.	i.	.....	.....	occurs in animal oils.
Coccerin.....	463.48	93	.....	.....	oil.	i.	s.s.	s.s.	occurs in cochineal "insects"
Convulmarin.....	298.3	49.5	.....	.....	.....	.....	.....	.....	occurs in Jalap and is a violent purge.
Commarin.....	154.06	.....	.....	.....	col. need.	s.s.	v.s.	v.s.	is the cis form of hydroxybenzamin.
$\alpha$ -Cumarin.....	154.06	308	d.	.....	col. need.	s.s.	v.s.	v.s.	occurs in leaves of Melilotus.
$\alpha$ -Crotolin.....	85.05	73	185	0.873 (73)	col. monoclin.	8.3	.....	s.h. pet. eth.	occurs in soil and croton oil and is very irritating and toxic.
Cysic.....	42.02	.....	.....	1.140 (4)	col. monoclin.	d.	.....	.....	an explosive occurring in blood (less than 1 mg./100 cc.) perhaps an intermediate in urea formation.
Cyanuric.....	139.05	>360	.....	1.708 (0)	col. monoclin.	0.58 (27)	0.33c.	v.s.s.	a polymeric of cyanic acid found in urine or formed on heating of urea.
Cyridine phosphoric.....	307.04	231 d.	.....	.....	.....	.....	s.	.....	a nucleotide from nucleic acid from cell nuclei.
Decylenic.....	170.14	1	149 (4)	.....	.....	.....	.....	.....	is a constituent of butter.
Dihydroxycholelin.....	302.31	190	.....	.....	.....	.....	s.	.....	a constituent of bile.
Dipallic.....	322.04	300	.....	.....	.....	20	167	v.s.s.	occurs in galls on plants.
Dihydroxypalmitic.....	388	125	.....	.....	.....	.....	.....	.....	occurs in cod liver oil.
Dihydroxystearic.....	316.28	126.5	.....	.....	rhomb.	.....	.....	.....	occurs in castor oil and inferior oils, there are 10 isomers.

..... for 15.00 and 20.00 on 100 cc. of alcohol

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Acid									
Dodecanilamino-diaz-									
boric	286.21	255.4			oil. leaf.	i.	s.	s.	a synthetic amino acid.
Elaidic	282.27	51.5	288(100)	0.815(79)	oil. leaf.	i.	s.	s.	is the trans isomer of oleic a.
$\alpha$ -Eleostearic	280.3	49	285(75)		leaf.	i.	s.	v.s.	occurs in China wood (tung) oil.
$\beta$ -Eleostearic	280.3	71	285(12)		leaf.	i.	s.	v.s.	occurs in China wood (tung) oil.
Elagic	338.08	d.	840(unb.)	1.87(13)	yel. cryst.	v.s.s.	s.s.	i.	occurs in plants and is used in tanning.
Escarbin (Heptylic)	130.1	-10	235.5	0.825(50)					is formed by oxidation of the aldehyde.
Eruic	328.22	35.5	281(90)	0.805(54.4)	need.		v.s.		occurs in mustard and other oils.
Erucic	194.08	169	d.		rhomb. need.	v.s.h.	v.s.	s.s.	occurs in black fir resin.
Formic	46.02	8.4	101.5	1.218(20)	oil. liq.	misc.	misc.		occurs in acids; normal blood contains 10 mg. per 100 cc.; is increased in methanol poisoning.
Fumaric	337.84			1.23		s.	s.		a cellulose stain.
Fumaric	116.08	287	290	1.555	oil. grn.	9.8(100)	5.75(30)	0.72(35)	is an intermediate in metabolism and occurs in the fumigary Fumaric.
Gadoleic	306	24.5			need. fal.				from codfish.
Gallie	225.39	189-7							occurs in galls.
Glucic	156.1				syrup	s.	i.	i.	is derived from glucose and may be produced by fermentation.
Glutaric	147.08	206d.			oil. pl.	1(14) sh.	v.s.s.		an amino acid from proteins; an ingredient of ajinomoto, a Japanese flavor.
Glutaric	138.06	97.5	302d.		oil. monoc.	64(20)	v.s.	v.s.	occurs in sheep wool; is saponifiable.
Glyceric	106.06				syrup	misc.	misc.	i.(v.s. acet.)	is formed in alcoholic fermentation.
Glycolic	465.35	175			pris.	s.h.h.			an ingredient of bile composed of glycolic acid and fatty acid.
Glycolic	465.34	104			oil. need.	0.55 c.	v.s.	v.s.s.	an ingredient of bile.
$\alpha$ -Glycolic	78.03	59.0(79)	d.		leaf. f. eth.	s.	s.	s.	occurs in plants.
Glyoxylic	82.03				rhomb.	v.s.	s.s.	s.s.	occurs in uric acid fruit.
Guaric	362.17	150			need.	1	s.s.		a nucleotide from nucleic acid, from cell nuclei.
Haidroic	325.3	74			need.		s.		is said to occur in fern.
Heaocenic	356.4	99							occurs in peanut oil and tubercle bacilli.
Heaenic	116.06	32	217	0.955(20)			s.		is formed from lauric aldehyde which occurs in green leaves.
Hippuric	179.08	138	d.	1.571(21)	oil. rhomb.	53(20)	s.s.	s.s.	a detoxication compound of benzoic a. with glycine.
Homogentisic	168.06	145.5			need.		s.		occurs in alkaptonuria and is probably derived from tyrosine.
Hydrocyanic	329.22	50			leaf.		s.s.	s.s.	occurs in chauliogra oil and is used in the treatment of leprosy.
Hydroxyacetic	27.02	-14	28.0	0.87(13)	oil. liq.	misc.	misc.		an insecticide.
$\beta$ -Hydroxy butyric	104.06		130(14)	1.148(15)	syrup	s.	s.		is formed by partial oxidation of fatty a. in animals and is increased in diabetes.
Hydroxy citric	208.06	180		1.59(35)	need. $\alpha$ -H <sub>2</sub> O	v.s.	s.s.	v.s.	is found in sugar beets.
$\beta$ -Hydroxy glutamic	150.06	rac. 196d.			pris.	v.s.	i.	i.	an amino acid obtained by Dakin by butanol extraction of protein hydrolysate.
Hydroxyisovaleric	244.2	82				i.	s.		occurs in Aspicin.
$\gamma$ -Hydroxy phenylacetic	150.06	148			pc. need. fv.	v.s.h.	v.s.	v.s.	is derived from tyrosine in intestinal putrefaction.
Hydroxy-propyl-gly-									
thoxy glutamic									a dipeptide isolated by Dakin from liver extract.
$\beta$ -Hydroxypropionic	90.05		d.				s.		is a synthetic compound.
Hypogaeic	224.26	33	239(15)			i.	v.s.	s.	occurs in peanut oil.
Indole acetic	175.18	164			leaf.	s.h.h.	s.	s.	from putrefaction of tryptophan.
Indole lactic	211.1	99			need.	s.	s.s.	s.s.	from putrefaction of tryptophan.
Indoxy-sulfuric	235.17	130			pl.	s.	s.h.		from putrefaction of tryptophan.
Inosinic	345.13					s.h.	i.	v.s.s.	a nucleotide from nucleic acid from nucleoproteins from cell nuclei.
Isoic	220.15	41			leaf.		s.s.	v.s.s.	occurs in iso nut oil.
Juniperic	272.3	56				s.h.h.	s.	s.	occurs in juniper and arbor vitae.
$\alpha$ -Kynuric	180.06	523			need.	v.s.s.	s.h.	s.s.	a derivative of tryptophan in dog's urine.
$\beta$ -Lactic	90.06	27	d.	1.249	pc.	misc.	misc.	misc.	is derived from glucose in animal tissues.
$\beta$ -Lactic	90.06	18	125(15)	1.249	oil. syrup	misc.	misc.	misc.	is produced by fermentation of carbohydrates.
$\gamma$ -Lactic	90.06	26	123(15)		syrup.		s.		is formed from sugar by Bacillus acid lactic.
Lanolinic	454.48	105							occurs in lanolin.
Lauric	200.19	48	255(100)	0.883(30)	oil. need.	i.	v.s.	v.s.	occurs in spermaceti.
Lerulic	116.06	30.1	246d.	1.140(17)	leaf.	s.a.s.	s.s.	s.s.	may be derived from fructose, nucleic acid or glycoproteins.
Lignoceric	368.28	80.5			need. fal.	s.h. C <sub>2</sub>	s.	s.	occurs in sphingomyelin and lignite tar.
Linoleic	278.28	138	232(17)	0.914(30)	oil. liq.	i.	10		occurs in drying oils, 100 mg. of which per day prevent fat deficiency in rat.
Linolic	280.27	<-13	230(16)	0.903(30)	oil. yel. col.	i.	misc.	misc.	occurs in drying oils; its Ca soap is soluble in water.
Malic	116.06	130.5	135d.	1.560	oil. gr.	72.8(25)	68.9(30)	8(25)	is derived from malic a. and is a cis isomer of fumaric a.
Malic	134.05	100	d.140	1.565	oil. need.	v.s.	s.s.	s.s.	occurs especially in fruits and is an intermediate in metabolism.
Malonic	134.05	135.5	d.		oil. trid.	>100	s.	s.	occurs in plants.
Margaric	270.27	98.9	277(100)	0.893(30)		i.	s.s.	v.s.	occurs in lichens but is usually synthetic.
Mallic	452.48	91			oil. scale	i.	s.s.s.	v.s.s.	occurs in beewax.
Mellitic	342.06	287	d.		oil. need.	v.s.	s.		an ingredient of honeystone in pest and brown coal.
Methylacrylic	85.05	16	165.0	1.065	oil. pr.	s.	misc.	misc.	occurs in Roman camomile oil.
Methyl asenic	140.0	161			pl.	s.	s.		an antipneumonic essential.
	159.06	d.115		3.112	yel. hex.	s.s.			a reagent for phosphorus and used in reduction tests.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Acid									
Montanic	438.45	33			plates		s.h.		occurs in montan wax from brown coal.
Mucic	210.06	d.200			col. cr. powd.	0.33(14)	i.		is formed by the oxidation of $\beta$ -galactose.
Mucosinic sulfonic									a hydrolytic product of mucosins.
Mucosinic	142.05	d.230				0.02	s.l.s.	s.l.s.	is derived from myricic acid.
Myristic	228.32	58.0	259.5(100)	0.858(30)	col. leaf.	i.	v.s.l.s.	v.s.l.s.	occurs in various fats; human subcutaneous fat contains 1%.
Nervonic	356.53	41			col. need.		s.	s.	occurs in nervon and sphingomyelin.
Nicotinic	122.05	332	subl.		col. need.	s.h.	s.h.	v.s.l.s.	occurs in plants.
Nondecylic	268.3	66.5	299(100)		l.f.f.	i.			said to occur in fats.
Oleic	282.37	14	286(100)	0.865(18)	col. need.	i.	misc.	misc.	occurs in oils.
Oxalidic	118.06	176d.			need. f. aces.	s.	s.	s.	from hydrolysis of a glucoside in lichens.
Oximinic	254.9	30	100	3.39	monocl.	s.	s.	s.	a reagent for fixation of tissues in histology.
Oxaloacetic	132.02	74	137						is an intermediate in metabolism.
Oxalic	120.05	101	subl.	1.538	col. monocl.	9.5(15)	20.7(15)	23.0(15)	occurs in plants and animals; precipitates Ca and prevents blood clotting and causes tetany.
Palmitic	256.26	64.0	239-240	0.858(32)	col. need.	i.	9.3(20)	s.	occurs in fats, forms 20% of human fat.
Palmitolic	282.23	47	246(15)		col. need.	i.	v.s.	v.s.	occurs in oils and Japan wax.
Pancreas myelin									the prosthetic group of the amylase.
Parasorbic lactone	112.1	136	221	1.063					occurs in arctic oil and produces vomiting.
Pectic	494.2					i.	i.		from action of acids on pectin, a plant jelly.
Pelargonic	186.14	13	254	0.907(30)	liq.	v.s.l.s.	s.	s.	occurs in pelargonium.
Pentadecylic	242.33	54	257(100)				s.	s.	occurs in Agaveae.
Petroselinic	282.25	34		0.862(40)	col. leaf.				occurs in parsley seeds.
Phenylacetic	136.06	78.7	265.5	1.238	col. leaf.	v.s.h.	v.s.	v.s.	is produced by intestinal putrefaction and is antiseptic, 5 g. is toxic to man.
						s.l.s.			
$\beta$ -Phenylpropionic	180.13	49	280	1.071(49)	need.	s.l.s.	s.	s.	is changed to benzoic a. in the body, the lethal dose is 100 g.
Phenocetic	284.38	105							occurs in phenosin in the brain.
Phthalic	166.05	101d.		1.586(30)	col. rhomb.	0.54(14)	11.7(13)	0.50	a dye intermediate.
						11.0(36)			
Phytachic	254.94	28			star	i.	s.	s.	occurs in tallow.
Phytic	660.42	214		1.175	amor.	s.l.s.	s.l.s.	s.l.s.	formed by action of acid on phytin.
Picric	226.05	121.3	exp.	1.787(19)	y. leaf. f.w.	1.23(20)	5.6(15)	1.06(13)	a reagent used to precipitate proteins and for creatinine.
						6.33(100)			
Polydipalmyl- $\beta$ -keto-									
digallic									a tannin.
Propionic	74.05	-22	141.1	0.992(30)	col. liq.	misc.	misc.	misc.	occurs in fermentations.
Protocatechuic	172.06	196d.		1.542(4)	monocl.	s.	v.s.	s.	occurs in shepherd's-purse.
Pseudoparic									a hypothetical isomere of cypaic a.
Pyrogallacrylic	494	95			i.	s.h.	s.		occurs in pygma wax.
Pyruvic	88.05	13.6	156 s.d.	1.397(30)	col.	misc.	misc.	misc.	is an intermediate product in metabolism of proteins, fats, and carbohydrates.
Quinic	182.1	169.0	d.	1.037	col. monocl.	40(9)	s.	v.s.l.s.	
Ricinoleic	298.27	17	250(15)	0.945(15)	color.	i.	misc.	misc.	occurs in castor oil; its alkali soaps are very soluble and detoxify antigens.
Ruberythric	554.22	360			y. need.	s.l.s.	v.s.	i.	a glucoside of alizarin in madder roots.
Sabitic	215.2	64				s.l.s.	v.s.		occurs in juniper and arbor vitae.
Saccharic	210.05	lactone		d.		v.s.	v.s.	s.l.s.	is formed by the oxidation of $\beta$ -glucose.
Salicyclic	183.05	139	subl.	1.443	col. need.	0.19(20)	30.00(13)	50.00(15)	derived from wintergreen, an analgesic used in rheumatism and colds.
Sebacic	262.14	127	255(100)		thin col. leaf.	0.1(17)	v.s.	v.s.	is produced by oxidation of fats.
Sorbic	112.06	134.5	228d.		col. need. f.w.	s.l.s.	v.s.	v.s.	occurs in mountain ash berries.
$\beta$ -Sorbic lactone	112.1	139	221	1.063		s.l.s.	s.	s.	a cathartic from mountain ash berries.
Stearic	284.39	69.3	350(100)	0.847(16)	color.	i.	2.5c.	v.s.	occurs in fats.
Suberic	174.11	140	279(100)		col. need. or tab.	0.14(15)	s.	v.s.l.s.	is derived from suberin in bark.
Succinic	118.05	135	235	1.552	col. monocl.	6.8(30)	s.l.s.	s.l.s.	occurs in plants and animals; muscle oxidizes it to fumaric a.
Tartaric	200.3	50.5							occurs in Guatemalan Turril seeds.
Tartaric	130.05	140		1.806	col. tab.	120(20)	v.s.	v.s.l.s.	occurs in plants and is nephrotoxic.
Tauromalic							s.	i.	an ingredient of bile formed of tartaric, deshydrouricolic a., and fatty a.
Tauromalic	533.43				delic. need.	v.s.	s.	s.l.s.	an ingredient of bile.
Telluric	280.3	6	225(13)	0.9429(20)					occurs in East African gourd seeds ( <i>Telfairia</i> ).
$\alpha$ -Termeosanic	338.37	65.5							occurs in peanut oil.
Tetradecanoic	226.21								1.4% in whale oil.
Tetrahydroxy stearic									
Stearic	284								occurs in <i>Streptanthus</i> oil and is a purgative.
Thapsic	386.34	124			i.	s.	s.l.s.		occurs in <i>Thapsia persica</i> .
Thioacetic	50.08	5	200d.		liq.	misc.	v.s.	v.s.	occurs in saliva and other body fluids.
Thymicic	354.15	235							a nucleotide from nucleic a. from nucleoproteins from cell material.
Thymus nucleic					i.	i.	i.	i.	from nuclei of cells in thymus gland (an organ of childhood).
							s.	s.	occurs in citron oil and is the isomere of angelic a.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
<b>Acid</b>									
Triacetic acid	176.06	161.3	d.	.....	col. rhomb.	40.5	v.s.	s.l.s.	occurs in beet molasses.
Trichloroacetic acid	214.3	51	236(100)	0.849	pl.	i.	v.s.	v.s.	occurs in figs and coconut oil.
Trihydroxyacetic acid	312	146	.....	.....	.....	.....	.....	.....	is a purgative.
Undecylic acid	186.17	29.3	228(150)	0.779	scale	i.	s.l.s.	s.l.s.	occurs in castor oil.
Uric acid	146.06	d.	1.859	.....	scale	0.005	i.	i.	the end-product of purines of muscle and cell nuclei.
Uridic phosphoric acid	340.14	206	.....	.....	.....	.....	.....	.....	a nucleoside from uracil, a, from nucleoproteins, from cell nuclei.
Uroacetic acid	124	224	.....	.....	.....	s.l.s.	s.l.s.	v.s.l.s.	a pimaric acid derived from histidine and found in dog's urine.
Valeric acid	118.06	-145(-20)	186	0.849(20)	liq.	2.7(16)	misc.	misc.	occurs in valerian.
Vanillic acid	182.08	181	subl.	.....	cr. f. w.	1/2100(14)	v.s.	v.s.	occurs in vanilla seeds.
Xanthic acid	255.18	.....	.....	.....	.....	i.	.....	.....	a nucleoside from uracil, a, from nucleoproteins, from cell nuclei.
Yeast nucleic acid	.....	.....	.....	.....	.....	i.	.....	.....	from nucleoproteins from yeast nuclei.
Ascorbic acid	176.12	150	.....	.....	pr.	33	4.5	2.35	an ascorbic alcohol from ascorbic acid in tocopherol.
Ascorbic acid	176.12	150	.....	.....	col. leaf or need.	v.s.l.s.	v.s.	v.s.	0.04-0.05 cured typhoidosis in young mice; it is more toxic in sunlight.
Ascorbic acid	176.12	150	.....	.....	.....	s.l.s.	.....	.....	50 cc. of 1% solution has been injected in human veins for African sleeping sickness.
Ascorbic acid	176.12	150	.....	.....	.....	.....	.....	.....	a sugar used also as a test for glycerol from which it is derived by heating and dehydrating.
Adenine	135.08	365	.....	.....	need. f.w.	0.09 cold.	s.l.s.	i.	a purine base derived from uracil acid.
Adenosine	267	229	.....	.....	need.	s.	s.l.s.	.....	adenine riboside, a nucleoside from uracil acid.
Adonitol	182.08	102	.....	.....	.....	.....	.....	.....	occurs in Adonia.
Adrenaline	183.16	307	315.5	.....	need.	s.l.s.	s.l.s.	i.	the hormone of the adrenal cortex stimulating sympathetic innervated muscle.
Alanine	89.06	297	367.2	.....	rhomb. cr.	20	v.s.l.s.	i.	an amino acid from proteins, yielding glucose in diabetes.
Alanyl alanine	180.113	270	.....	.....	need.	s.l.s.	i.	.....	a dipeptide.
Alkaloids	88.08	.....	88(20)	1.108(20)	liq.	1/2	s.	s.	is a condensation product of acetaldehyde and perhaps an intermediate in fat synthesis.
Alloxan	240.06	260	480	.....	cr. need.	i.e., s. alk.	v.s.	v.s.	is formed by hydrolysis of ruberythrin, a; stains new bone tissue.
Alloxanic acid	242.13	.....	.....	.....	.....	.....	.....	.....	is a biological stain.
Alloxan	188.06	265	.....	.....	wh. cryst.	0.6c, v.h.	v.s.l.s.	i.	an oxidation product of uric acid found in animals and plants.
Allose	180.09	.....	.....	.....	.....	.....	.....	.....	a synthetic sugar (hexose).
Alloxan	142.08	250.5	.....	.....	col. pr.	v.s.	s.	.....	a toxic pyrimidine derivative occurring in tissues; if bottled it sometimes explodes.
Alloxan	248.06	170.5	.....	.....	wh. cr. powd.	v.s.	v.s.l.s.	v.s.l.s.	a condensation product of alloxan intermediate in the nucleoside test for uric acid.
Allyl alcohol	58.05	-129	97.0	0.864(20)	liq.	misc.	misc.	misc.	an antiseptic in 0.5% solution; 100 times as toxic as ethanol of which it is an impurity.
Allyl disulfide	146	.....	158	0.9(15)	.....	.....	.....	.....	occurs in garlic.
Allose	180.09	.....	.....	.....	.....	.....	.....	.....	a synthetic hexose sugar.
Aluminate	58.97	656.7	1800	2.71	octah.	i.	i.	i.	toxic for plants in soils below pH 4.7 and above 8, AlCl <sub>3</sub> paralyzes sweat glands.
Ammonia	17.03	-77.70	-33.35	0.7220(0/1)	gas.	cold H <sub>2</sub> O 89.9(8) hot H <sub>2</sub> O 7.4(100)	13.2(30)	s.	a globulin (protein) from almond and peach seeds.
Ammonia	17.03	-77.70	-33.35	0.7220(0/1)	gas.	cold H <sub>2</sub> O 89.9(8) hot H <sub>2</sub> O 7.4(100)	13.2(30)	s.	arises from the decarboxylation of proteins and forms urea, some of which reforms NH <sub>3</sub> in kidney.
Amniotin	457.22	320	.....	.....	rhomb. f.w.	8.3 mg. (10)	0.11 mg. (10)	i.	β-mandelonitrile glucoside from bitter almond kernels yielding HCN.
Amniotin	180.11	.....	147.6	0.870(14)	liq.	0.18(30)	misc.	misc.	occurs in essential oils.
Amniotin	88.10	-78.5	137.9	0.817(14)	col. liq.	2.7(22)	misc.	misc.	27 times as intoxicating as ethyl alc.
Amniotin	88.10	-12	128	0.816	col. liq.	.....	.....	.....	27 times as intoxicating as ethyl alc. produced by fermentation.
Amniotin	138.09	245	.....	.....	.....	.....	.....	.....	a synthetic anesthetic derived from barbituric acid.
Aniline	93.06	-6.2	184.4	1.022(20)	liq.	3.1(16)	misc.	misc.	an ingredient of coal tar causing methemoglobin formation and anemia.
Asiatic acid	186.06	2.05	247	1.123	col. liq.	s.l.s.	misc.	misc.	occurs in Tahiti vanilla.
Asiatic acid	186.06	2.05	247	1.123	liq.	i.	s.	.....	is used to kill lice.
Asiatic acid	186.06	2.05	247	1.123	col. leaf.	i.	0.38(15)	1.17(15)	is toxic to fish and vegetation around coaling plants; a photosensitizer.
Asiatic acid	186.06	2.05	247	1.123	pale y. need.	i.	2.3(70)	v.s.l.s.	is purgative and contained in Caesarea agave.
Asiatic acid	186.06	2.05	247	1.123	hex. rh.	i.	i.	i.	trivalent 10 times as toxic as pentavalent, used in trypanosome diseases.
Asiatic acid	186.06	2.05	247	1.123	leaf.	100	100	2.3	a synthetic antipyretic.
Asiatic acid	186.06	2.05	247	1.123	y. leaf.	.....	.....	.....	a flavone dye formed by hydrolysis of apigenin.
Asiatic acid	186.06	2.05	247	1.123	need.	s.l.s.	i.	i.	apigenin glucoside in celery and parsley leaves.
Asiatic acid	186.06	2.05	247	1.123	wh. amor.	s.l.s.	1(60%)	s.; s.l.s. HCl	is a methyl tetraose constituent of apigenin, a glucoside in celery and parsley leaves.
Asiatic acid	186.06	2.05	247	1.123	.....	.....	.....	.....	an anesthetic derivative of morphine; dose 5-10 mg. hypodermically.
Asiatic acid	186.06	2.05	247	1.123	rhomb.	56(10)	v.s.l.s.	i.	arabinose-polyaccharide occurring in the mottles of <i>Albugo matricariae</i> .
Asiatic acid	186.06	2.05	247	1.123	col. warts	v.s.	v.s.l.s.	.....	is a pentose sugar obtained by hydrolysis of gum arabic.
Asiatic acid	186.06	2.05	247	1.123	.....	.....	.....	.....	occurs in algae liquor.
Asiatic acid	186.06	2.05	247	1.123	.....	.....	.....	.....	a histon (protein) from <i>Arctia</i> (see uricin) sperm.
Asiatic acid	186.06	2.05	247	1.123	need.	v.s.l.s.	v.s.	i.	hydroquinone glucoside from arbutin leaves and pear trees used as a diuretic.
Asiatic acid	186.06	2.05	247	1.123	pr. f. w.	18(21)	i.	i.	an amino acid in proteins; it is essential in nutrition.
Asiatic acid	186.06	2.05	247	1.123	.....	.....	.....	.....	an antipyloric arsenical.
Asiatic acid	186.06	2.05	247	1.123	.....	.....	.....	.....	trivalent more toxic than pentavalent, 200 mg. lethal, used in syphilis.
Asiatic acid	186.06	2.05	247	1.123	.....	.....	.....	.....	an antipyloric arsenical.
Asiatic acid	186.06	2.05	247	1.123	.....	.....	.....	.....	a p-cyanine derivative from American wormwood used to free the gut of worms.
Asiatic acid	186.06	2.05	247	1.123	.....	.....	.....	.....	an antipyloric arsenical.
Asiatic acid	186.06	2.05	247	1.123	.....	.....	.....	.....	an alkaloid from belladonna, stimulates respiration, dilates bronchi, depresses gut, stops secretion of a vital stain.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Aurania.....	456.13								used for light filters and biological stains.
Aureolin.....						i.			a globulin from oats.
Bactital.....	184.11					0.88(20)	s.	v.s.	a synthetic anesthetic derived from barbituric acid (malonyl-urea).
Barium.....	137.36	870	1140	3.80(20)	y. metal	d.cov. H <sub>2</sub> O.			may partially replace Ca but paralyzes respiratory center. BaCl <sub>2</sub> 0.5 g. per kg. is fatal.
Benzocholin.....						i.	d.	i.	is a vitamin in frog's eggs where it is probably combined with lecithin.
Benzocaine.....									a protein in urine of patients with myeloma; it coagulates at 80° and redissolves at 100°.
Benzocaine.....									is a component of atropine (glucoside in bitter almonds); is a local anesthetic.
Benzaldehyde.....	106.06	-50.0	179.5	1.049	oil liq.	0.38	misc.	misc.	
Benzene.....	78.05	5.5	79.60	0.878(20)	oil. rhomb.	0.07(22)	misc.	misc.	
Benzidine.....	184.11	138.7	401.7		lust. scale f.f.w.	0.94(100)	s.	2.2	is a vasodilator and causes anemia.
Benzoin.....	212.1	133	344		her. f.al. sl.a.h.	a.h.			an antiseptic not found in gum benzoin.
β-Benzopropene.....	102.08	48.5	306	1.108(20)	rhomb.	i.	13.8(18)	17.3(18)	a mild hypnotic.
Benzopyrrolin.....	726.4								is used as a biological stain.
Benzyl alcohol.....	108.06	-15.3	205.8	1.048(20)	oil liq.	4(17)	misc.	misc.	occurs in balsams; is a diuretic and anesthetic (local).
Benzyl benzoate.....	212.1	18.5	324	1.114(13)	oil. leaf. or liq.	i.	s.	misc.	occurs in balsams.
Benzyl bromide.....	170.97	4.0	199.0	1.498(44)	liq.	i.	misc.	misc.	a tear gas.
Beryllium.....	9.02	1300	1850(5)	1.85(20)	her.	i.	s.		may replace 1/3 of the Ca in Ringer-Locke fluid.
Betaine.....	117.1	270d.			oil. monocl.	v.s.	s.		a sweet alkaloid in tests and animals.
Bilirubin.....	612.32	182-2.5			dk. r. rhomb.	v.s.; s. alk.	s.	v.s.	the bile pigment derived from hemoglobin and coloring the eyes in jaundice.
Biliverdin.....	624.32				black pryd.	v.s.; s. alk.	s.	s.	a green pigment resulting from oxidation of bilirubin.
Bismuth.....	208.00	271.0	1470(1495-1550)	9.80(20)	her.	i.	i.	i.	insoluble salts cast X-ray shadows, soluble salts very toxic, used in syphilis.
Niuret.....	121.08	130			oil. need.	45.5(100) 1.54(3)	v.s.	v.s.	a condensation product of urea having the peptide linkage and giving the biuret test.
Borneol.....	184.14	210.5	subl.	1.011	oil. her. leaf.	v.s.	v.s.	v.s.	occurs in Borneo and Sumatra camphor and essential oils.
Bromine.....	216.13	590			yl. need.	s.	s.	s.	a red liquid dye.
Brilliant cresyl blue.....	389.61								a blood stain.
Brilliant green.....	402.9								an indicator for bacteriological media.
Bromochlor phenol blue.....	381.28								an indicator with pK <sub>a</sub> = 6.2.
Bromocresol green.....	630.26								an indicator with pK <sub>a</sub> = 4.7.
Bromocresol purple.....	540.41								an indicator with pK <sub>a</sub> = 6.3.
Bromide of Na.....	107.91	755	1180	3.205	sub. oil.	121(100)	s.		blood contains 1 mg. Br per 100 cc., 4 g. NaBr depressant to adult, 10-15 g. causes edema.
Bromoderm.....	322.75	7.7	180.4	2.960(30)	oil liq.	s.	misc.	misc.	is used as a local anesthetic in whooping cough; dose 0.2 cc.
Bromobenzene.....	672.75								an indicator.
Bromophenol blue.....	422.38								an indicator with pK <sub>a</sub> = 6.2.
Bromophenol red.....	422.38								an indicator with pK <sub>a</sub> = 6.2.
Brom thymol blue.....	624.21								an indicator with pK <sub>a</sub> = 7.0.
Buht camphor.....	168.12	82	110		need.	s.	s.		a terpene oxidation product from Dorenia leaves (S. Africa) dose 3 g. leaves.
Butanol.....	74.08								a fermentation product of carbohydrates used in extracting amino acids.
Butyraldehyde.....	72.08	-89.0	115.7	0.830(20)	oil liq.	3.7	misc.	misc.	occurs in essential oils.
γ-Butyrolactone.....	145	120	220		leaf.				a ptomaine.
Bypass.....									protein threads attaching the cell to muscle (Myofibrils) to rocks.
Carbolic oxide.....	226.95	-35	110	1.491(15)	i.				an antipyrilic essential.
Carbavene.....	103.13	9	179-9	0.885(15)	liq.	s.	s.		a ptomaine occurring in body fluids and purifications; deacetylated lysine.
Cadmium.....	112.41	320.9	778	8.660(20)	monocl.	i.	i.	i.	4% CdSO <sub>4</sub> disinfectant.
Caffeine.....	194.11	287		1.53(15)	wh. need.	1.53(15); 45.5(10)	2.3(16) (85%)	0.044(16)	occurs in tea and coffee, therapeutic dose 0.2-0.5 g., toxic dose 1 g., lethal dose 10 g.
Calcium.....	40.08	810	1430-5(1170)	1.55	sub. wh.	d. to Ca(OH)	s.		blood plasma contains 10 mg. per 100 cc., 50% diffusible, 25% indiff.; lack causes osteoporosis.
Camphene.....	136.13	30	160	0.822	fash. need.	i.	v.s.	v.s.	occurs in essential oils.
Camphor.....	152.13	179.0	209.1	0.960(25)	her.	v.s.	130(10)	v.s.	occurs in the camphor laurel, its value as a heart stimulant is doubtful.
Canavanin.....									a globulin (protein) of the jack bean.
Cantharidin.....	194.1	212			rhomb. pl.	i.	0.08(18)	0.11	the active principle of Spanish flies causing blisters; dose 0.25-0.5 mg.
Capric aldehyde.....	184.21		61(15)		liq.				occurs in essential oils.
γ-Carboxy aldehyde.....	128.17		60(16)	0.838(15)	liq.				is found in citron.
Carbon dioxide.....	44	-89.6 (5.2 atm.)	-80	1.83 (air)(15)	gas	179.67(10)	319.9(15)	s.	partial pressure in blood nearly 40 mm. Hg. stimulates respiratory center.
Carbon tetrachloride.....	153.83	-33	76.8	1.495(20)	oil liq.	0.08(20)	misc.	misc.	an antihelmintic; dose 0.05 cc. per kg. for hookworm.
Carbonyl hemoglobin.....	16.665								a compound of hemoglobin and CO; death does not occur until over 80% of Hb is COHb.
Caranubyl alcohol.....	354.39	89			leaf.	s.	s.		a constituent of caranuba wax and woolfat and gives the latter its water-absorbing qualities.
Carminin.....	359					s.	s.		a nitrogenous base extracted from muscle.
Carosine.....	226.14	240				31(25)			β-alanyl histidine a dipeptide found in meat.
Carotene.....	538.6				yl.				a yellow pigment in yellow and green vegetables that is a substitute for vitamin A.
Caryophyllin.....	180.11	0.5	238	0.979(20)	oil	s.al.	misc.	misc.	is a vitamin (protein) in carp eggs where it is probably combined with lecithin.
Caryov.....	152.11		235	0.968(20)		v.s.	misc.	misc.	a vermifuge and fungicide from caraway seed.
Casien.....									a terpene from turpentine used in perfumes.
Castoreum.....									a phosphoglycerin is the chief protein of milk and cheese, isoelectric point pH 4.7.
Catechol.....	110.08	104	145	1.344	leaf.	v.s.	v.s.		a globulin from European chestnut.
Cellose.....	942	190	d.			v.s.	i.	i.	occurs in essential oils, is antiseptic.
									a bi-enzyme obtained by partial hydrolysis of cellulose.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Cerium.....	140.13	940	1400	cub. 0.92(35) hex. 6.70	cub. or hex. gray met.	i.	i.	i.	oxide used to allay vomiting, soluble salts very toxic, used to catalyze ashing of food.
Ceryl alcohol.....	332.43	80	.....	.....	colorl.	i. (s. sat.)	s.h.	30(35)	a constituent of animal and vegetable waxes and wool fat.
Cerium.....	132.81	35.5	.....	1.90(20)	hex. sil-wh.	d.	s.h.	.....	may replace half the K in animal tissues,
Cetyl alcohol.....	242.37	45.3	.....	0.819(30)	leaf. f.al.	i.	s.	a.	from spermaceti.
Chalcidol.....	179	48	200	.....	rhomb.	s.	.....	.....	a mustard oil derived from a glucoside in wallflower seeds.
Chelidonium.....	332.2	130	.....	.....	.....	i.	s.	s.	an alkaloid from opium and Chelidonium depresses smooth muscle.
Chloral hydrate.....	165.4	47.4	56.0d.	1.508	col. tab.	66	v.s.	.....	a hypnosis causing fatty infiltration of the liver when used in excess.
Chloramine T.....	212.7	.....	.....	.....	.....	s.	.....	i.	an antiseptic in 0.5-5% solution; lethal dose less than 1 g. per kg.
Chlorate of Na.....	106.45	345	d.	2.49(13)	col. trig. col.	79.0(0)	s.	.....	a weed eradicator.
Chloroacetal green.....	520.31	.....	.....	.....	.....	.....	.....	.....	an indicator with $pK_a = 4.3$ .
Chloride of Na.....	58.45	804	1413	2.163	rub. col.	35.7(0)	s.h.	.....	blood plasma contains 0.8% but 1% is approximately isosmotic (physiological salt sol.).
Chloroform.....	119.38	-63.5	61.2	1.489	col. liq.	0.62(21)	misc.	misc.	an anesthetic causing fatty infiltration of the liver when used in excess.
Chlorophyll a.....	916	106	.....	.....	.....	s.	s.	.....	the green coloring matter in plants.
Chlorophyllin a.....	.....	.....	.....	.....	.....	.....	.....	.....	a dicarboxylic acid derived from chlorophyll a.
Chloroplatin.....	164.4	-64	112.4	1.692(0)	liq.	i.	misc.	misc.	an anesthetic was gas and insecticide.
Chloroquin.....	420.39	.....	.....	.....	.....	.....	.....	.....	an indicator with $pK_a = 8$ .
Chlor phenol red.....	336.34	145	>300	1.067(20)	monoc. tab.	i.	20h.	18	gives the animal cell surface its waterproof properties; forms gall-stones.
Cholesterol.....	386.64	.....	.....	.....	visc. liq.	s.	s.	.....	an alcohol or pimarane occurring in body fluids and plants and combined in lecithin.
Choline.....	121.13	.....	.....	.....	.....	s.	s.	.....	used as an indicator in determination of chloride with silver nitrate.
Chromate of Na(H <sub>2</sub> O) <sub>2</sub> .....	342.16	13.93	.....	1.493	monoc. yel.	s.s., misc.	s.h.	a.	a dye intermediate.
Chromones.....	146.05	89	.....	.....	need.	i.	s.	a.	a flavone dye in poplar buds.
Chrysine.....	254.08	275	subl.	.....	leaf.	1/130	s.h.	.....	an alkaloid occurring in cinchona bark related to quinine, lethal dose 8 g.
Chrysotene.....	294.19	354.3	.....	.....	col. need.	0.027(20)	1	0.27	an analgesic derived from quinine and used in opiat.
Cinchoygen.....	261.1	.....	.....	.....	.....	s.h.	.....	.....	a fungicide oxidation product of terpine from turpentine.
Cineol.....	154.19	.....	177	0.929(35)	.....	i.	v.s.	v.s.	an alkali occurring in cinchona bark related to quinine, lethal dose 8 g.
Cinnamic aldehyde.....	132.06	-7.5	53(30)	1.05	col. liq.	v.s.h.	misc.	misc.	an itch remedy from oil of cinnamon.
Cinnamyl cocaine.....	330.19	121	.....	.....	need.	i.	s.	.....	an alkaloid found in coca leaves.
Cis-larvin.....	154.14	104	358	.....	.....	i.	s.h.	s.h.	an ingredient of cough syrup.
Citronellal.....	154.14	.....	508	0.859	col. liq.	v.s.h.	misc.	misc.	occurs in citronella oil and is related to terpenes.
4-Citronellol.....	156.16	.....	321.7	0.857(35)	col. liq.	v.s.h.	misc.	misc.	occurs in citronella oil and is related to terpenes.
Clupein.....	.....	.....	.....	.....	.....	.....	.....	.....	a proteinase (protein) from herring sperm.
Cobalt.....	58.94	1480	2990	8.90	rub. blue	i.	.....	.....	a metal related to iron and contained in the body in small amounts.
Cocaine.....	300.17	98	.....	.....	col. monoc.	0.14(24)	20(25)	26.3	an alkaloid from coca leaves; a local anesthetic, dose 15 mg., lethal dose 1.3 g.
Cococeryl alcohol.....	454.48	104	.....	1.1088(14)	white	misc.	misc.	misc.	occurs in cochinilla wax.
Codine.....	317.19	155 sat.	179	1.215(14)	col. orthorh.	0.83(25)	62.5(25)	8(35)	an optically active alkaloid used particularly to check coughing, therapeutic dose 80-90 mg.
Cod Intestinal.....	.....	.....	.....	.....	.....	.....	.....	.....	is a vitamin (protein) in cod eggs where it is probably combined with lecithin.
Collagen.....	.....	.....	.....	.....	.....	.....	.....	.....	the chief protein of bones, tendons, and white fibrous connective tissue.
Coccaravulin A.....	.....	.....	.....	.....	.....	i.	i.	i.	a globulin (protein) of jack beans.
Coccaravulin B.....	.....	.....	.....	.....	.....	.....	.....	.....	a globulin (protein) of jack beans.
Coccolithin.....	710.8	.....	.....	.....	.....	i.	i.	i.	the protein of the shells of bivalve molluscs and their eggs.
Conglutin.....	.....	.....	.....	.....	.....	.....	.....	.....	a globulin (protein) from almonds and lupine seeds.
Congo red.....	1072.33	.....	.....	.....	.....	.....	.....	.....	is used as an indicator and biological stain and test for cellulose.
Coumarin.....	176.19	159	d.	.....	need.	s.h.	s.	i.	methoxyhydroxymethyl alcohol glucoside in oranges and other plants.
Crocin.....	127.14	-2.5	165.5	0.945(20)	col. liq.	l.h.	misc.	v.s.	an alkaloid from hemlock; lethal dose about 1.1 g. due to respiratory paralysis; antidote adrenal.
Copper.....	63.57	1083	2310	8.92	cub. relati. met.	i.	i.	i.	occurs in the body in small amounts, its lack may cause anemia in young.
Coprosterol.....	388.37	110-14	.....	.....	.....	i.	s.h.	18	is an isomer of dihydrocholesterol formed by reduction of allocholesterol in the gut.
Corylin.....	.....	.....	.....	.....	.....	.....	.....	.....	a globulin from hazel nuts.
Cresatin.....	146.05	70	361.7	0.933	col. rhomb.	v.s.h. (s.h.)	v.s.	v.s.	an adulterant of vanilla.
Creatine.....	133.1	265	.....	1.33(1)	col. monoc.	0.098(70)	i.	.....	an amino acid found in muscle where it is probably exists as phosphocreatine.
Creatinine.....	113.08	360d.	.....	col. prism. f.w.	.....	8.7(15)	0.16c. abs.	.....	the anhydride of creatine; the creatinine in urine per day is constant in 1 person.
Cresol.....	.....	.....	.....	.....	.....	.....	.....	.....	a proteinase (protein) from <i>Cresidura</i> (wren) sperm.
α-Cresol.....	108.08	12	202.8	1.033	col. liq.	2.4(35)	misc. >80	misc. >10	is used as an antiseptic and is less toxic than p-cresol.
α-Cresol.....	108.06	30.1	190.8	1.048	colorl.	3.1(35)	misc.	misc.	is antiseptic and an ingredient of trisectol in liquor cresolis compositus, U.S.P.
p-Cresol.....	108.06	34.5	202.8	1.033	col. prisma.	2.8(40)	misc. >10	misc. >10	is derived from tyrosine in the gut, is antiseptic, lethal injection 0.8 g. per kg.
Cresolphthalein.....	344.11	.....	.....	.....	.....	.....	.....	.....	an indicator with $pK_a = 9.4$ .
α-Cresol purple.....	332.27	.....	.....	.....	.....	.....	.....	.....	an indicator.
Cresol red.....	332.27	.....	.....	.....	.....	.....	.....	.....	an indicator with $pK_a = 8.4$ .
Cresyl violet.....	332.33	.....	.....	.....	.....	.....	.....	.....	a vital stain for blood.
Cryptopyrrol.....	120.11	.....	65(23)	0.93	.....	i.	s.	s.	one of the 4 pyrrol derivatives of hemin and chlorophyll.
α-Crystallin.....	.....	.....	.....	.....	.....	.....	.....	.....	forms 37% of soluble proteins of lens of eye.
β-Crystallin.....	.....	.....	.....	.....	.....	.....	.....	.....	coagulates at 60° (3° lower than α-crystallin).
Crystal violet.....	407.72	116	.....	.....	hex. gem.	1.6(26)	14(26)	.....	is a Gram stain for bacteria; 5 mg. per kg. intravenously as antiseptic.
Cuscutyryne.....	944	.....	169(23)	0.976(17)	.....	misc.	misc.	misc.	an alkaloid from cuscuta leaves of Peru.
Cutin.....	80	.....	.....	.....	.....	.....	.....	.....	a waterproof insoluble oil and acid acetate of human keratin

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Cyanamide.....	42.03	44	160(19)d.	1.083	col. need.	v.s.	v.s.	v.s.	is said to accelerate certain oxidations.
Cyanidin.....	304.1				a.	a.	a.	i.	the aglycone of many anthocyan glycosides which give color to flowers and fruits.
Cyanin.....	444.8	304			rhomb.	v.s.s.	v.s.s.		is cyanidin glycoside, a detoxication compound coloring flowers and fruits.
Cyloxyterin.....	240.33	353d.				a.	i.	i.	a protamine of sperm of lamp fish not hydrolyzed by pepsin HCl.
Cymene.....	134.11	-75.5	176	0.86(18)	col. liq.	i.	v.s.	a.	is an antiseptic agent of lamp fish not hydrolyzed by pepsin HCl.
Cyprinin.....									is an antiseptic agent of lamp fish not hydrolyzed by pepsin HCl.
$\beta$ -Cyprinin.....									is a protamine from the sperm of the carp.
Cysteine.....	121.12				cr. powd.	v.s.	a.		is a reduction product of cystine, an amino a. found in proteins and urinary calculi.
Cytine.....	240.33	353d.			reg. hex. pl.	0.01	i.	i.	an amino a. found particularly in kerner; essential to life and nephropathic.
Cytidin.....	245.13	220			need.	s.s.			cytosine-riboside, hydrolyzed by intestinal juice.
Cytochrome.....									a heme pigment found in all cells and related to respiration enzymes.
Cytosine.....	111.07				plates	s.s.			a pyrimidine derived especially from thymus nucleic a.
Delphinidin.....	330.1				br. cryst.	a.	a.	i.	the aglycone of an anthocyan dye in flowers.
Delphinin.....	392.8	303d.			violet				the glycoside of delphinidin occurring in larkspur.
Derran.....	(182.5) <sub>6</sub>					s.			a glucosin causing rages of wies.
Dextrin.....	(182.18) <sub>6</sub>			1.038	wh. amor.	v.s.h.	i.	i.	a name applied to hydrolytic products of starch of higher m.w. than maltose.
Dibutyrin.....	301.02	116			need.				p-hydroxy-mandelaldehyde glycoside found in millet and sorghum.
$\alpha$ -Dibutyryl.....	179.09	40	176(40)	1.178( $\frac{1}{2}$ )		misc.	v.s.	a.	is formed by action of pancreatic lipase on tributyrin.
Dandel blue.....	841.4								a vital stain.
Darsinone.....	282.14	260							a disaccharide of arabinose.
Dibenzylglyoxalose.....	330	145			need.				a glycoside found in <i>Dioscorea latifolia</i> .
Dibromotolide.....	318.48				purple				Tyrian purple, occurs in Mower and <i>Porphyra</i> .
$\alpha$ -Dibutyryl.....	232.15	<-40	352	1.303	oil		misc.	misc.	is formed by the action of pancreatic lipase on tributyrin.
Diochloramine T.....	240	83				i.	a.		is a more powerful antiseptic than chloramine T.
$\alpha$ -Dichloro benzene.....	146.85	-17.5	179	1.268(20)	colord. liq.	i.	a.		is used as an insecticide, sometimes dissolved in gasoline.
p-Dichloro benzene.....	146.85	33.9	170	1.458	lowl. f.l.	i.	a.	a.	is used as an insecticide, sometimes dissolved in gasoline.
Diochloro furanose.....	401.01								is used as an indicator in titrating chlorides with $AgNO_3$ in neutral sol.
Dibromate of $Na_2H_2O_4$ .....	286.05	(-1H <sub>2</sub> O 100) (anh. 330)	400d.	2.33(13)	monocr. r. deliq.	260(10) (anh. 450(80))	i.		is toxic and is used as a mordant in dye industry and in tanning of leather.
Diethyl disulfide.....	122.21		133.5	0.832(20)		v.s.s.			is toxic to <i>Aecyris limbricoides</i> .
Digitalin.....	700.43	217				1/1000	150%	v.s.s.	lethal dose 1 cat unit per kg. body wt., is used to slow the heart-rate.
Digitalose.....									a methyl pentose constituent of digitalin.
Digitonin.....	1188.74	253d.			need.	i.	s.s.	s.s.	is a hemolytic glycoside of digitals used to precipitate cholesterol.
Digitarin.....	822.56	34			need.	s.s.	s.	s.s.	is a glycoside of digitals leaves, 1 mg. stimulating heart as much as 1 g. dry leaves.
Dihydrocholesterol.....	416.55								is formed from cholesterol in the body and excreted in the gut.
Dihydrocholesterol.....	388	144			hex. pl.				occurs in plants.
Dihydroxy acetone.....	90.05	75			colord.	a.	s.s.		a triose that is converted by alkali into fructose.
Dioxytyrosine.....	432.69	205				s.s.	s.s.		an amino acid found in argemone, spongia, and thyroglobulin.
Dimethylamine.....	45.06	-98.0	7.4	0.680(10)	gas.	v.s.	a.	a.	a protamine used to attract boll weevils to exterminate them.
Dimethyl aniline.....	121.1	1.87	133.5	0.856(20)		v.s.s.	a.	a.	a yellow liquid.
Dimethyl guanidine.....	87				need.				is a vasodilating protamine.
Dimethyl-pheno-sulfonin.....	350.64								is a nuclear stain.
Dimethyl sulfate.....	126.11	-21.8	138.5	1.302( $\frac{1}{2}$ )	col. liq.	v.s.s.		a.	is very toxic.
$\alpha$ -Dinitrobenzene.....	168.05	50.7	302	1.544(17)	need. f.l.	0.01a.	3.5(20)	v.s.	lethal dose 0.5 g. per kg.
$\alpha$ -Dinitrobenzene.....	168.05	118.5	319	1.36(20)	tab. f.l.	0.38(100)	3.8(25)	v.s.h.	is used as an indicator for oil oxidation-reduction.
p-Dinitrobenzene.....	168.05	172.1	299	1.655(20)	need.	0.18(100)	0.4(30)	s.br.	is toxic.
2,4-Dinitrophenol.....	194.04	154			y. need. f.w.	s.s.	v.s.h.	v.s.	is a H <sup>+</sup> indicator - lethal dose 0.5 g. per kg.
2,4-Dinitrophenol.....	194.05	111.6		1.683(20)	y.s.l.w.	a.h. 4.3(100)	s.s. 3.9(50)	v.s.	is a H <sup>+</sup> indicator - lethal dose 0.5 g. per kg.; a metabolic stimulant.
2,4-Dinitrophenol.....	194.05	61.8			y. need. f.w.	v.s.h.	v.s.h.	v.s.	is a H <sup>+</sup> indicator - lethal dose 0.5 g. per kg.
2,4-Dinitrophenol.....	194.05	134			need.				is a H <sup>+</sup> indicator - lethal dose 0.5 g. per kg.
2,6-Dinitrophenol.....	194.05	120.1			leaf.				is a H <sup>+</sup> indicator - lethal dose 0.5 g. per kg.
D-nucleoside (adenine- uracil).....	650					a.	i.		is formed by the action of pancreatic juice on yeast nucleic a.
$\alpha$ -Dulcin.....	321	0		0.920(21)				a.	is formed from triolein by the action of lipase.
$\alpha$ -Dulcin.....	338	67					a.		is formed from tripalmitin by the action of pancreatic lipase.
Diphenylchlorarsine.....	294.5	89	333	1.883(40)		i.	v.s. s.h.	v.s.	sweat gas, produces necrosis of skin and fatty infiltration of liver.
$\alpha$ -Diterpin.....	624.36	74.5						a.	
Dopa, 3,4-dihydroxy- phenylalanine.....	197.14	285d.				a.		v.s.s.	is oxidized by the action of certain oxidases to dark-colored compounds.
Dulcitol.....	182.11	138	298(5.5)	1.466(15)	col. pr.	del.; v.s.h.	v.s.s.	v.s.s.	is a C atom sweet alcohol.
Echinoderm.....						a.		a.	is a red pigment in sea urchins.
Ecdysterin.....						i.	i.	i.	is slightly denatured ecdysterin.
Ecdysterin.....	20,000					i.	i.	i.	a globulin of hump and cottonseed, deficient in cysteine and lysine.
Egg albumin.....	33,800					a.	i.	i.	is crystallizable as ovalbumin, 0.00016 mg. is said to be sensitive (anaphylaxis).

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Ethoxy alcohol.....	288.34	70	220				s.l.a.	s.l.a.	is one of the constituents of sebum and palm wax of Madagascar.
Elastin.....						i.	i.	i.	the protein of yellow elastic tissue digestible by trypsin.
Emetine.....	308.34	74			need.	0.1	v.a.	v.a.	is derived from ipecac, 50 mg. emetine HCl is injected subcutaneously for amebic dysentery.
Emodin.....	270.08	250			or. monoc. pr.	a.	s. glc. acet. a.		cathartic action of sennae is due to liberation of emodin from glucoside.
Enilatin.....	538.13				di.pr.				occurs as glucoside coloring black grape skins.
Enin.....	538.6				di.pr.	a.			glucoside of enilatin coloring black grape skins.
Eosin (yellowish).....	447.74				red need.	i.	s.	s. acet. h.	an acid stain for tissues.
Epinephrine.....	185.13	40	255d.		col. cryst.	a.	s.	a.	an alkaloid from <i>ma huang</i> with adrenaline action, used in asthma.
Ergosterol.....	382.83	159-3		1.04		i.	s.	s.	a sterol abundant in fungi.
Erythritol.....	122.06	126	531	1.451 (20)	tetrag.	v.a.	s.l.a.	v.s.	a 4 C atom sweet alcohol occurring in lichens.
4-Erythrose.....	120.1					v.a.	s.		a 4 C atom aldose (sugar).
5-Erythrose.....	120.1								a 4 C atom aldose (sugar).
6-Erythrose.....	120.06								a 4 C atom aldose (sugar).
Esculin.....	340.13	205 an.h.	230d.		wh. need.	s.	s.		a 4 C atom ketose (sugar).
Eserin.....						sh.	4.58 (70)	v.a.l.a.	a glucoside of eseretin from horse chestnut, lethal dose 4 g., protecting vs. ultraviolet.
Ethanol.....	46.06	-114	78.4	0.789 (20)	colorl.	misc.		misc.	a protamin from sperm of pike, white fish, and lake trout.
Ethyl acetate.....	88.06	-83.6	77.1	0.899 (20)	colorl. liq.	8.8 (20)	misc.	misc.	causes intoxication when concentration in blood is 0.04-0.7%.
Ethyl amine.....	45.06	-80.6	16.9	0.699 (4)	colorl. liq.	misc.	misc.	misc.	10 cc. per kg. is lethal for dogs; it is an anesthetic.
Ethyl bromide.....	189.56	-119	58	1.49 (20)	colorl. liq.	0.09 (20)	misc.	misc.	is a paraffine.
Ethyl butyrate.....	116.09	-98.3	121.3	0.879 (15)	colorl. liq.	0.68 (25)	s.	s.	8 cc. with re-breathing is used as an anesthetic for tooth extraction.
Ethyl chloride.....	84.5	-138.7	12.3	0.81 (20)	colorl. liq.	2.00	misc.	misc.	artificial pineapple flavor (odor).
Ethyl ether.....	74.08	-118.3	34.5	0.714	colorl. liq.	8.3 (17.5)	misc.		4-18 cc. inhaled produces general anesthesia; sprayed it produces local anesthesia.
Ethyl formate.....	74.08	-50.5	54.3	0.932	liq.	11	a.	a.	used as a general anesthetic since 1942.
Ethyl hydrocupressate.....	340.55	123.8				i.	s.	s.	4-6 cc. are given as a hypnotic, it has a peach kernel odor.
Ethyl iodide.....	155.97	-108.5	72.2	1.993	liq.	0.4 (20)	s.	s.	the therapeutic dose for pneumonia, 1.5 g. per day may produce blindness.
Ethyl mercaptan.....	61.11	-121	24.7	0.640 (20)	liq.	1.5	a.	a.	0.4-1.3 cc. are inhaled for short analgesia.
Ethyl propionate.....	102.08	-72.6	59.1	0.891	colorl. liq.	2.4 (20)	misc.	misc.	gives a bad odor to feces.
Ethylene.....	28.05	-169.4	-169.3	0.978 (air)	gas	26.4 (0)	360 cm <sup>3</sup>	a.	is used as a general anesthetic and to ripen fruits. Troops due to gas leaks prevent blooming of flowers.
Ethylene chloride.....	98.95	-33.3	69.7	1.257	colorl. liq.	s.l.a.	a.	misc.	50 vols. to 10 vols. of O <sub>2</sub> produces general anesthesia.
Ethylene oxide.....	44.03	-111.3	10.7	0.897 (7)	colorl. liq.	misc.	misc.	misc.	is used to poison soil to kill bacteria.
Etiophyllin.....	457.38	205			b. tabl.		a.	a.	in the decarboxylated tetrapyrrol—Mg base of etiophyllin, the green plant pigment.
Etioporphyrin.....	455.26	200			violet		s.h.	a.	is a Mg-free tetrapyrrol derivative of etiophyllin and said to have been derived from heme.
Eucelatin.....	144.78					i.	i.	i.	is a globulin in Brazil nuts.
Eye albuminoid.....						i.	i.	i.	constitute 50% of the protein of the crystalline lens.
Fenchone.....	152.13	6	185	0.944	cryst.	i.	v.s.	v.a.	is an isomer of camphor in fennel.
Fibrin.....						i.	i.	i.	is a combination of fibrinogen and thrombin or fibrinogen and tissue fibrinogen.
Fibrinogen.....						i.	i.	i.	is a blood plasma globulin probably formed in the liver and taking part in blood clotting.
Fibrin.....						i.	i.	i.	is an indigestible protein of silk containing diisopropylamines in its molecule.
Fissalin.....	288.208	300			y. need.				is the aglucone of the glucoside, fustin, the yellow dye of young fustic.
Flavone.....	222.08	57			need.				is the colorless base of the color of many flowers.
Flavosavin.....	322.00	260d.			or. powd.	i.; a. alk.	a.	a.	2% in 5% NaHCO <sub>3</sub> is used in the diagnosis of corneal injury.
Fluorine.....	38.00	-223	-187	1.89 (18 air)	s.v. gas.	d.			tooth enamel may contain 0.5%. Drinking water saturated with CaF <sub>2</sub> causes mottled teeth.
Formaldehyde.....	30.02	-92	-21	0.815 (-20)	col. gas.	a.	a.	a.	green leaves in sunlight give timonol test; 100 cc. 40% HCHO lethal; forms methylene epox.
Frasin.....	370.14	190			need.	s.h.	s.		a glucoside of fraxetin in bark of ash tree.
4-Fructose.....	180.1	104	1.699 (17.5)		need. f.w.	v.s.	50	a.	is 1.73 times as sweet as glucose; is absorbed more slowly than glucose from the gut.
Furural.....	166.11	153							is a 4 C atom sweet alcohol with the addition of a methyl group.
Fucose.....	184.09	145				5.07			is a methyl pentose occurring in the seaweed, <i>Fucus</i> .
Furfuraldehyde, furfural.....	94.03	-58.7	161.7	1.159	colorl. liq.	9 (13)	a.	a.	is derived from pentoses; lethal dose for rabbit 0.8 cc. per kg. body weight.
Galactan.....							i.	i.	a polysaccharide yielding galactose on hydrolysis.
4-Galactose.....	180.09	168			het. tabl.	v.a.	s.l.a.		as sweet as sucrose, normal tolerance in man 50 g. in women 40 g. stored as galactosides.
Gastrin.....	314	190	120d.			s.l.a.	s.l.a.	i.	is methyl salicylate glucoside in wintergreen and yellow birch.
Gelatin.....						s.h.	i.	i.	is formed by boiling collagen in water, it is deficient in tyrosine, tryptophan and cystine.
Genianose.....	504.26	209							is a trimethoxide in gentian.
Geniobiose.....	342	186				v.s.	a.		is a glucose-glucoside in mother liquor in glucose manufacture or by partial hyd. of genianose.
Gentianin.....	344.06	207	400		need.	v.a.l.a.	s.l.a.	s.l.a.	is a xanthone dye from gentian.
Gesneral.....	154.14	<-15	229	0.881	colorl. liq.	i.	misc.	misc.	an open-chain terpin aldehyde from lemon grass oil.
Germanium.....	72.6	588.5	(2700)	5.35 (4)	cubic met.	i.	i.	i.	is said to increase erythrocyte formation.
Glibit.....						i.	a (75%)	i.	a polkamin constituting 40% of protein of wheat flour, also in rye, durum, Einkorn, emmer, spelt.
Globin.....						i.	i.	i.	a histon constituting 83.5% of hemoglobin.
Globulin from Cotton seed.....						i.	i.	i.	the chief protein of cottonseed and is adequate for growth.
Pumpkin seed.....						i.	i.	i.	only 38.4% of amino acids from hydrolysis ascertained for.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Globulin from									
Wheat flour						i.	i.	i.	may be due to admixture of embryo of bean.
$\beta$ -Glucosamine	176.11	110d.			symp.	v.s.a.	i.	i.	is an amino-sugar from hydrolysis of chitin and glycoproteins.
$\alpha$ -Glucose H <sub>2</sub> O	180.16	146			need. f.al.	80(17.5)	s.a.	i.	0.1% in normal blood, after fasting 12 hrs. and ingesting 50 g. is high in blood for 1-3 hrs. may yield equimolecular proportions of lactic acid and phosphoric a. in muscle.
Glucose phosphate	278.16					v.s.	i.	i.	living tissue contains 25-150 mg. per 100 cc. it is a hydrogen acceptor in dehydrogenation.
Glycerin		190d.							
Glycerin from									
Corn						i.	i.	i.	is very small in amount.
Cute						i.	i.	i.	
Wheat						i.	i.	i.	
Glyceric aldehyde	90.05	138				s.a.	v.s.a.	v.s.a.	is usually called glycerin, forms 40% of proteins of wheat flour.
Glycerol	92.08	17.9°	260		colorl.	liq. misc.	misc.	i.	a triose sugar, utilizable but somewhat toxic.
Glycine	75.05	333d.			monool.	23	v.s.a.	i.	a constituent of ordinary fats, converted into glucose in the diabetic.
Glycinin						i.	i.	i.	an amino acid synthesized in the body, abundant in gelatin.
Glyogen	162.08%	240			wh. amor.	v.a.	i.	i.	the globulin of soy beans of high biological value.
Glyoxylin									the storage carbohydrate of animals and yeast, the human body contains about 800 g.
Glycol	62.05	-17.4	197.5		colorl. liq.	misc.	misc.	s.a.	a plant constituent with insulin-like action.
Glycol aldehyde	60.03	97			glass	v.a.	v.s.h.	s.a.	a C atom sweet alcohol, said not to be converted into glucose in diabetic.
Glyoxal	58.02	15	50.4		1.14	v.a.	s.	s.	the simplest sugar and is converted into glucose in the body.
Gold	197.2	1.063	2.800		19.23(17.5)	reg.	i.	i.	is combined with NH <sub>3</sub> to form glycine by glyoxalase.
Gorgonin						i.	i.	i.	although used in medicine, gold salts are very toxic.
Guanidine	58.06				cryst.	v.a.	v.a.	v.a.	a heteroprotein from gorgonins (sea fans) yielding 9% di-iodo-tyrosine.
Guanine	151.08	360d.			col. need.	i.; s. alk.	v.s.a.	v.s.a.	normal blood contains only about 0.1 mg. per 100 cc. It is difficult to separate from creatine.
Guanosin	262.14	237d.							a purine which is transformed into uric acid in the body.
Guanosinic acid	362.17	180				i.			guanine pentoside from pancreas uric acid.
Guaiaol	124.05	58.3	305		1.14( $\frac{1}{4}$ )	col. prisms	1.6(15)	s.	guanine phosphoric acid.
Halloprotein									an aromatic antiseptic from guaiac and beechwood tar and crocote.
Hematin	300.09	250d.			br. pl.	0.6(30)	s.a.	s.	is a glycoprotein from the small, <i>Elater</i> .
Hematin	592.13	184d.			br. powd.	s. alk.	s.h.	i.	is a dye formed by oxidation of hematoxylin used as a stain and for analysis of heavy metals.
Hematoxylin	597.5	104d.			dk. v. rhomb.	i.	s.	s.a.	is the prosthetic group of hemoglobin; for spectroscopy must be paired with protein.
Hematoxylin	595.19	140			tetrag.	v.s.a.	s.	s.	a photosensitizer, tetragonal formed from hemoglobin by the action of certain drugs.
Hemin	627.98								is a dye precursor found as glucoside in logwood and on oxidation yields hematin.
Hemoglycin	22,70%					0.09			is the crystalline prosthetic group of hemoglobin, said to be hematin HCl.
Hexadecyl alcohol	172.19	13	229		0.827(23)				is the blue, O <sub>2</sub> -carrying Co-compound in the blood of molluscs and some other animals.
Henricinone	426.40	68.1	360(15)		0.781(82)	leaf.			is found in oil of rose.
Heparin	626								a paraffin found in various waxes.
Hepatosine	360.43	59.5	270(15)		0.779(90)				a glycosaminic acid compound from liver; injection of 100 mg. prolongs coag. time of blood 3 times.
Hepyl alcohol	114.12	-34.6	176.8		0.8185	col. liq.	0.26(100)	misc.	a paraffin found in waxes.
$\alpha$ - $\beta$ -Esteric aldehyde	98.08		49(17)		oil				an anesthetic and anesthetic.
$\alpha$ -Esteric alcohol	102.11	-36.1	155.6		0.82(33)	col. liq.	s.	misc.	a constituent of green leaves.
Heryl resorcinol									occurs in fruit oil and some essential oils.
(apophenol)	114	58	178(5)		y. liq.	s.a.	s.	s.	does 0.15-0.6 g. 3 t.p.d. for urinary antiseptic.
Histamine	111.1					s.		i.	a very toxic substance when injected lowers blood pressure and increases gastric secretion.
Histidine	155.09	27d.			pl.	s.	i.	i.	an essential amino acid abundant in histones, globin containing 1%.
Histone from									
Chicken erythrocyte						s.	i.		is probably globin.
Goose erythrocyte						s.	i.		is probably globin.
Snake erythrocyte						s.	i.		is probably globin.
Codfish sperm						s.	i.		is not digested by pepsin, 2.8% histidine, 15.8% arginine, 8.3% lysine.
Flounder sperm						s.	i.		
Lotus vulgaris sperm						s.	i.		is not digested by pepsin, 2.8% histidine, 12% arginine, 8.1% lysine.
Shad sperm						s.	i.		
Tum. ood sperm						s.	i.		
Horden						i.	s.	i.	a protein of low biological value from barley.
Hordenine	116	117.5	174(11)		colorl.	s.	s.	s.	a base from barley embryos; 30-40 mg. hypotermically arrests diarrhea in dogs.
Hydrazine	32.05	122			col. gr.	0.035(30)	0.74(25)	0.8(25)	an alkaloid from hydrazine, lethal dose 0.1 g., therapeutic dose 0.01 g.
Hydrogen ion	1					s.	s.	s.	are depressant to nerves except the respiratory center and large amounts on cutaneous nerves.
Hydroquinone	110.05	170.5	268.2		1.329(15)	3.9(15)	v.a.	v.a.	lethal dose 20 g. is used in photography and chemical analysis for its reducing action.
Hydroxy cholesterol	402.37					i.	s.	s.	is said to play a part in the absorption of fats.
Hydroxyethylamine	61.06		171		1.022	liq.	s.	s.a.	is a constituent of kaphalin acid and is produced by putrefaction of serine.
Hydroxy proline	121.08	270d.				v.a.	v.a.	i.	an amino acid found in proteins.
Hygrine	127		198		0.868(17)	br. oil	s.	s.a.	is a pyridine alkaloid.
Hyoscyamine	289.19	106.0			need.	5.0	v.a.	v.a.	is the isomere of atropine and more effective on the iris.
Hypoxanthine	136.06	150d.			need.	0.07(34)	1.4(100)	s.	is hydroxy purine and occurs in the body.

	M.W.	M.P.	R.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Icthyolipidin.....						i.			a protein of fish scales intermediate between collagen and keratin.
Idiose.....	180.09								a sugar isomeric with glucose.
Indican.....	249.19	57 m.h.	d.			v.a.	v.a.	s.	is indoxyl glucoside in indigo plant, by hydrolysis and oxidation, indigo blue.
Indigo carmine.....	465.19				blue powd.	s.	s.l.s.		is Na-indigo disulfonate a, a blue dye and plasma stain.
Indigo blue.....	262.09	305d.			monocl.	i.	i.	i.	is a blue dye insoluble in water but soluble in chloroform and CCl <sub>4</sub> .
Indol.....	117.06	51.5	254	1.0648 (90)	col. leaf.	s.h.	v.a.	v.a.	a ptomaine from putrefaction of tryptophan, lethal dose sub. cu. 0-5 mg.
Indol-ethylamine.....	180.11								decarboxylated tryptophan, injection raises blood pressure.
Inosin.....	268	213			need.	s.h.	i.		hypoxanthin riboside.
Inositol.....	180.09	204-47	250 (vac.)	1.793	rhomb.	v.a.h.	i.	i.	occurs in animals and plants, stimulates yeast growth.
Intervin.....						s.	s.		pancreas hormone causes storage of glycogen, burning of sugar, epileptiform seizures.
Inulin.....	990.84	178d.		1.85	micr. cr.	0.001 (15)	s.h.	s.	odd C fat for diabetes causes increase in sugar but not of $\beta$ -hydroxybutyric a.
Iodate of Na.....	197.92	d.		4.277 (90)	rhomb.	94 (100)	v.a.		a fructosan of Jerusalem artichoke tubers, hydrolyzed by HCl but not diastase.
Iodine.....	253.84	114	158.00	4.930	rhomb.	0.039 (20)	20.5 (15)	20.4 (17)	is used as yeast food, is toxic, oxidizes I <sup>-</sup> to I <sub>2</sub> in acid solution.
Iodine green.....	594.55								is in high concentration in seaweed, marine invertebrates, and thyroid and hypophysis, de- ficiency causes goiter.
Iodoform.....	280.3	119 (sub.)		4.1	y. hex.	0.01 (25)	1.3 (18)	15.9 (26)	is used to stain lignified xylem in plant tissues.
Iridium.....	193.1	2440 $\pm$ 5	4,400	22,421	cub.	i.	i.	i.	clotted on wounds increases phagocytosis, large doses internally are toxic.
Iron.....	55.84	1,535	3,000	7.58	cubic	i.	i.	i.	is used to coat hydrogen electrodes.
Ironce.....	122.16		144 (16)	0.509	col. liq.	v.a.s.	v.a.	v.a.	is necessary to mammals as a constituent of hemoglobin, but is necessary for chlorophyll form.
Iscamyl alcohol.....	88.1	-117.2	190.5	0.813	col. liq.	3.3 (20)	misc.	misc.	the violet odor found in orris root, a terpene.
Iscamylamine.....	87.11		85	0.747	col. liq.	v.a.s.	misc.	misc.	is the chief amyl alcohol of fermentation (fuel oil).
Iscamylamine.....	117.99		99	0.873 (90)	liq.	v.a.s.	misc.	misc.	a ptomaine causing fall in blood pressure, from putrefaction of leucine.
Iscamylamine.....	117.99		99	0.873 (90)	liq.	v.a.s.	misc.	misc.	causes vasodilation, dose 0.3 cc. of 50% in alcohol.
Iscamylalcohol.....	74.98	-108	107.30	0.802	col. liq.	3.5 (14)	misc.	misc.	is produced by fermentation.
Iscamylamine.....	73.39	-85.5	68	0.736	col. liq.	misc.	misc.	misc.	arises from putrefaction of valine and is a toxic ptomaine.
Iscamylamine.....	389.36	133					s.	s.	from wool fat, said to have been found in plants.
Iscamylamine.....	387	142					s.	s.	is formed from ergosterol.
Iscamylamine.....	122.11								is said to be 2-methyl hemopyrrol in which case it is not an isomer.
Iscamylamine.....	342.17								is hydrolyzed with difficulty and excreted in the urine unchanged.
Iscamylamine.....	131.11	260 c.s.d.			leaf.	4.0 (15.5)	1; s.l.s.h.	1.	is $\beta$ -methylerythritol- $\alpha$ -amino propionate a.
Iscamylamine.....	155.1		106						from pomegranate root, physiological action same as pallesticine.
Iscamylamine.....	141.1		107						from pomegranate, resembles pallesticine.
Iscamylamine.....	68.06	-120.0	94.0	0.879	col. liq.	i.	misc.	misc.	a hamiterpene, the unit of structure of terpenes, rubber, carotene, vit. A, phyol.
Iscamylamine.....	69.06		92.8	0.789 (90)	liq.	misc.	misc.	misc.	20 cc. is intoxicating.
Iscamylamine.....	249.17								a condensing disaccharide yielding glucose.
Iscamylamine.....	69.08		92.5	0.830 (9)	liq.	s.l.s.	s.	s.	occurs in essential oils, has apple odor.
Iscamylamine.....	333.19								is used as an intravital stain.
Iscamylamine.....									a globulin in walnuts and butternuts.
Iscamylamine.....									a phosphatide essential to blood clotting.
Iscamylamine.....									a galactoside of the brain.
Iscamylamine.....									an indigestible protein constituent of epidermis, hair, horn, nail, cornea, feathers, tortoise shell
Iscamylamine.....									from the egg shells of fish, reptiles, and monotremes.
Iscamylamine.....									an indigestible scleroprotein lining birds' gizzards, soluble in boiling water.
Iscamylamine.....									the albumin of milk of high biological value.
Iscamylamine.....	200.19	201.8 m.h.	d.	1.535 (20)	col. rhomb.	17c; 4th.	i.	i.	glucose-galactoside, milk sugar 10% as sweet as sucrose, tolerance 10 g., in pregnancy 25 g.
Iscamylamine.....	128.9	810	6.155		gray	det.			occurs as a catalyst in acting biological material.
Iscamylamine.....	357.83	89							from opium, produces tetanus like strychnine.
Iscamylamine.....	337.33	337.43	1,013	11.285 (1)	cub.	i.	i.	i.	0.04-0.26 mg. per day in urine in lead poisoning, remainder excreted by gut or stored in bones.
Iscamylamine.....	777.68	d.					v.a.	v.a.	a phosphatide component of surface layer of all living cells, important in fat metabolism.
Iscamylamine.....							s.	i.	an albumin of peas and beans.
Iscamylamine.....							s.	i.	a globulin of peas and beans.
Iscamylamine.....	131.11	265	1.183 (20)			2.4 (22)	0.1th.	1; (10.5 gins.	a widely distributed amino acid, skin contains 57%.
Iscamylamine.....							ac. a.)		
Iscamylamine.....							s.	i.	an albumin of wheat, rye, and barley embryos.
Iscamylamine.....	132.00				amor.	s.h.	i.	i.	lichen starch, digested by invertebrates only.
Iscamylamine.....	779.49								is used for staining cellulose.
Iscamylamine.....	404.559								lipin-cellulose.
Iscamylamine.....	128.13	-91.9	177.0	0.842 (20)	col. liq.	i.	misc.	misc.	an antiseptic terpene in essential oils, causing fatty infiltration of liver, excreted as glycuronate.
Iscamylamine.....	247.144	141			w. need.		s.		acetoxyphenyl glucoside from flax and <i>Platanus latifolia</i> .
Iscamylamine.....	6.94	136	1400	0.534 (20)	gray	det.			a solvent for uric acid, lethal dose of 1.0G for cats and dogs 60 mg. per kg. per day.
Iscamylamine.....	285.19	121			amor.	s.l.s.	v.a.	v.a.	an alkaloid from <i>Lobelia</i> with nicotine-like action.
Iscamylamine.....	269.18	330							tetrahydroxy, flavone dyestuff from woad ( <i>Rassia luteola</i> ).
Iscamylamine.....	146.13	234d.			f. need. f.w.	v.a.	v.a.s.	i.	the only straight-chain amino acid that does not form glucose in the diabetic.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Magnesium	24.32	651	1110	1.74	hex. sil. wh.	i.; d.h.	.....	.....	ion depresses nervous system, deficiency causes osteoporosis, $MgSO_4$ acts as osmotic cathartic.
Magnesium ethyl bromide	213	.....	.....	.....	.....	.....	.....	.....	Chignard's reagent is used in organic syntheses.
Maltose	342.10	.....	.....	1.540	fine need.	v.a.	v.a.s.	.....	a disaccharide 3% as sweet as sucrose, is more slowly absorbed from the gut.
Maltivitin	384	.....	.....	.....	.....	.....	.....	.....	the blue gluconate of the gluconic malvin of the wild mallow.
Manganese	54.93	1390	1900	7.30	rust. gr. pink wet.	dec.	.....	.....	1 mg. per 100 kg. blood plasma is the co-enzyme of laccase, it catalyzes oxidations with $O_2$ .
Mannan	132.06	.....	.....	.....	.....	.....	.....	.....	mannose polysaccharide in malar, ivory nut, seaweed and other plants, probably fermented in gut.
D-Mannitol	132.11	166.1	295(3.5)	1.499(20)	col. need.	15.6(18)	v.s.s.s. abs.	i.	a C atom sweet alcohol.
D-Mannose	132.09	133	.....	1.539	col. prism.	550	v.s.s.s.	i.	yield gluconic, is absorbed very slowly by the gut, occurs in plants, derived from mannan.
Mannosylase	594.3	150	.....	.....	colori. rh.	.....	.....	.....	glucose-glucose-phosphate from ash manna is indigestible by man.
Maytin	.....	.....	.....	.....	.....	s.	.....	.....	a globulin (2.8%) in corn meal coagulates at 70°.
Melanin	1779	.....	.....	.....	.....	.....	.....	.....	a black protein soluble only in alkali formed from white protein by oxidation of ring in tyrosine.
Melibiose	342	35-40	.....	.....	.....	.....	.....	.....	glucose-glactoside from yellow mallow.
Melittoside	324.23	149-50	.....	.....	rhomb.	33.3	.....	.....	glucose-glucose-fructose in sap of cinders and poplars, bees feeding on it die of starvation.
Menthol	156.18	35.5; 43.5	215	0.890( $\frac{4}{5}$ )	col. trim.	s.s.	v.a.	v.a.	mint camphor is aromatic, stimulates cold nerves, dose 40 mg. is vasodilator, hemolytic, anti-septic.
Merbaphen	551.28	.....	.....	.....	wh.	.....	.....	.....	nonaroid, 100 mg. intramuscularly causes diuresis due to liberation of Hg ions.
Mercurochrome	374.55	.....	.....	.....	brd. gr. scales	s.	.015	1. eth., chl.	an antiseptic, lethal dose 25 mg. per kg. in rabbits subcutaneously, is used externally and internally.
Mercuraphen	338.65	.....	.....	.....	.....	.....	.....	.....	sodium hydroxy-mercury ortho nitro phenolate.
Mercury	200.61	-38.89	356.90	13.548	silv. liq.	i.; s. $HNO_3$	.....	.....	a purgative and diuretic, diuresis due to poisoning convoluted tubules, very toxic to kidney.
Metaphen	793	.....	.....	.....	br. y. powd.	i.	.....	.....	an organic Hg. opt. used intramuscularly; 1/1000 solution is used in nose, eye, and urethra.
Metaproteins	.....	.....	.....	.....	.....	.....	.....	.....	.....
Acid	.....	.....	.....	.....	.....	i.	.....	.....	soluble in weak acid and alkali, not precipitated by heat.
Alkali	.....	.....	.....	.....	.....	s.	s.	.....	soluble in weak acid and alkali, not precipitated by heat, in formation, $NH_3$ and S split off.
Methane	16.03	-184	-161.4	0.555 (air)	gas	5.45 cm <sup>3</sup>	53.2 cm <sup>3</sup>	s.	is formed by fermentation and utilized by <i>Methanomonas</i> and <i>P. methanica</i> .
Methanol	32.03	-97.1	66	0.791(15)	liq.	misc.	misc.	misc.	is more toxic than ethanol because it is not metabolized, lethal dose 100-200 cc., blindness.
Methemoglobin	15.699	.....	.....	.....	br. y. need.	.....	.....	.....	is formed in the blood by nitrobenzene and other poisons and excreted in the urine.
Methionine	146.15	238.5	.....	.....	wh. hex. pl.	s.c.	s.	i.	$\gamma$ -methyl thiol- $\alpha$ -amino butyric acid from proteins.
Methyl acetate	74.08	-88.1	57.1	0.893	col. liq.	31.9(20)	misc.	misc.	is used in the determination of esterase.
1-Methyl-3-acetonyl- piperidine	152	.....	97(13)	.....	liq.	.....	.....	.....	is probably a vermicide.
Methylamine	31.08	-92.3	-5.5	0.699(-11.0)	gas.	1130 cm <sup>3</sup> (12)	s.	.....	a ptomaine from putrefaction of glycine and other substances, occurs in <i>Mercurialis</i> .
Methyl- $\alpha$ -amyl ketone	114.11	.....	130	0.822(15)	col. liq.	v.s.s.s.	s.	s.	is found in oil of cloves and Cayenne oil of cinnamon.
Methyl bromide	94.94	-93.0	4.5	1.753( $\frac{5}{8}$ )	gas	s.s.	v.a.	v.a.	formerly used as an anesthetic, now in refrigerating machines.
Methyl butyrate	126.08	<-95.0	102.3	0.858	col. liq.	s.	misc.	misc.	is used in perfumes.
Methyl chloride	50.48	-97.8	-23.7	0.890(13)	colori. gas	400 cm <sup>3</sup>	3600 cm <sup>3</sup>	v.a.	is used as a local anesthetic by refrigeration.
Methyl-ethyl ketone	72.08	-86.4	79.6	0.805(20)	col. liq.	s.	misc.	.....	occurs in wood alcohol and is used to precipitate paraffin from oils.
Methyl-ethyl pyrryl	108.066	.....	74-8(11)	.....	.....	v.s.s.s.	v.a.	v.a.	has been called homocypripod but is not isomeric with isohomocypripod.
Methyl formate	60.08	-69.8	31.8	0.875	col. liq.	30.4(20)	misc.	.....	is an odoriferous liquid.
Methyl glycol	72	.....	.....	.....	y. wh. oil	.....	.....	.....	is formed from glucose diphosphate by apurymase from tissues, and forms lactic a. with co-enzyme
Methyl green	457.17	.....	.....	.....	.....	.....	.....	.....	is used to stain mitochondria.
Methyl guanidine	75	.....	.....	.....	.....	.....	.....	.....	a ptomaine that may arise by putrefaction from creatine or arginine.
Methyl heptanone	126.11	-67.3	174(170)	0.890	col. liq.	i.	misc.	misc.	is found in essential oils.
Methyl- $\beta$ -heptyl ketone	149.14	-19	194-196	0.8317	liq.	i.	s.	s.	is found in oil of rose and oil of cloves.
Methyl iodide	141.99	-96.1	43.5	2.279	col.-br. liq.	1.4(20)	misc.	misc.	is an anesthetic, saturated water solutions are antiseptic against typhoid.
Methyl isobutyl ketone	170.18	15	224(220)	0.828(13)	colori.	i.	s.	s.	is found in oil of rose and oil of lime leaves.
Methyl orange	322.19	.....	.....	.....	cr. y. powd.	v.a.	s.	i.	is an indicator.
Methyl propionate	88.08	-87.5	79.9	0.817(20)	col. liq.	6.5(20)	misc.	misc.	is used in perfumes.
Methyl propyl ketone	88.08	-77.8	101.7	0.812( $\frac{4}{5}$ )	col. liq.	v.s.s.s.	misc.	misc.	is an impurity in alcohols.
Methyl red	260.14	.....	.....	.....	.....	.....	.....	.....	is an indicator from pH 4.4-6, is decolorized by reduction.
Methylxiphenolone	196.16	.....	.....	.....	.....	.....	.....	.....	is obtained from yeast.
Methylene blue	372.73	-215( $\frac{1}{2}$ ), 100	-325( $\frac{1}{2}$ ), 150	.....	grm. cr. powd.	s.	s.	.....	is hydrogenated to colorless opd. in body and hence a test of reduction; is a biological stain.
Methylene chloride	84.93	-96.7	40.1	1.329	col. liq.	2(20)	misc.	misc.	is anesthetic although toxic.
Metrazol	138	57-5	.....	.....	monocl.	v.a.	s.	s.	was thought to be a heart stimulant.
Monocrotin	134.08	.....	150(150)	1.20(13)	liq.	s.	s.	s.s.	is a solvent for basic dyes.
Monobutyrin	138.1	.....	271	1.008(17)	liq.	s.s.	.....	.....	has been synthesized with lipase.
Monoclecin	258.3	35	.....	0.847(21)	liq.	.....	.....	.....	has been synthesized with pancreatic lipase.
$\alpha$ -Monogalminin	320.3	72	.....	.....	.....	6.800% (22.5)	.....	.....	.....
$\beta$ -Monogalminin	320.3	74	.....	.....	wh. leaf.	.....	.....	.....	.....
$\alpha$ -Monocrocin	328.3	73.49	.....	.....	need.	.....	v.a.	s.	.....
$\beta$ -Monocrocin	328.3	80	.....	.....	.....	.....	.....	.....	.....
Moric	294.08	285	.....	.....	col. need.	0.085	s.	s. acet. s.	a flavone dye from fustic and orange orange.
Morphine	282.17	280.5	.....	1.217	col. need.	0.03	0.10	0.02	a narcotic alkaloid from opium.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological		
						Water	Alcohol	Ether			
Mucin, Frog egg									a glycoprotein yielding galactosamine on hydrolysis; it surrounds frogs' eggs.		
" Salivary									a glycoprotein containing mucosin-H <sub>2</sub> SO <sub>4</sub> ; it lubricates food swallowed.		
Mucoid, Chondromucoid						i.			a glycoprotein yielding chondroitin-H <sub>2</sub> SO <sub>4</sub> from cartilage.		
" Cornua									a glycoprotein.		
" Ovo						s.h.			forms 10% of solids of egg white.		
" Serum					powd.				forms 0.5-1% of serum proteins.		
" Tendon									yields galactosamine.		
" Umbilicus									is digested by pancreatic juice but not by gastric juice.		
" Virens tumor					wh. flocc.	i.			contains 1.19% S.		
Mucosamine	119.15				octahed.	s.	s.	i.	lethal dose 8 mg. subcutaneously; ptomaine from mushroom lowers blood pressure.		
Mustard gas	159				city liq.				penetrates tissues with intracellular HCl formation, hence used as war gas.		
Mustard oil	79.90				liq.	v.s.a.	v.s.	v.s.	is formed by hydrolysis of glucoside aliginin in mustard seeds; blisters skin.		
Myogen					1.85(20)				an albumin in muscle press juice that is said to change to a globulin myosin.		
Myosin									a globulin forming the clot in muscle press juice that forms on standing.		
Myristyl alcohol	433.48	88			oil. need. f.e.th.	i.	s.h.	s.	a solid monatomic alcohol of waxes.		
Myrrillin	222.113				0.777(85)				the aglycone of an anthraquin glycoside in stool, rose, and mallow.		
Myristin	194.11	116			1.335	rhomboh.			a methyl cyclohexane from the valve muscles of the black sea mussel, <i>Mytilus edulis</i> .		
Naphthalene	128.06	80.1			217.9	1.145	oil. monocl.	5.3 abs.	v.s.	is used to kill clothes moths and as an intestinal antispasmodic and vermifuge, dose 0.1-1 g.	
n-Naphthol	144.09	95			389	1.224(4)	yel. monocl.	s.h.	v.s.	is applied to skin in scabies and absorbed is nephrotoxic, causes exanthem, hemocidemia.	
Narcotine	402.37	170				oil. prism. f.v.	0.078(13)	0.1		lethal dose 0.4 g., a hypnotic alkaloid.	
Narceine	412.19	175				1.374	oil. need. f.al.	0.094(20)	1(20)	an opium alkaloid, lethal dose 1.3 g.	
Nearaphenamine	393				or. y. powd.	s.				the least toxic of amineals, therapeutic dose 300 mg. intravenous but 300 mg. per kg. is tolerated.	
Necine	158.144									a nitrogenous base of muscle.	
Nerol	130.18				102-4(12)	0.888(19)				a terpene from essential oils.	
Nervon	302.74	41					s.	s.	s.	a galactoside of the brain.	
Nevrine	103.4						s.	s.	i.	a ptomaine from putrefaction of choline, lethal dose 45 mg. per kg. animal, but 1 mg. per kg. toxic.	
Nicotinamide	122.12						i.	i.		an indigestible protein of brain more resistant to alkali than epidermal keratin.	
Neutral red	102.18					5.645(26)	2.45			a vital stain and indicator.	
Nickel	58.96	1463			3900	8.90	cup. sil. metal	i.		occurs in insulin; nickel carbonyl is a poisonous gas.	
Nicotina	180.11					1.078				an alkaloid similar to nicotine but more toxic.	
Nicotine	162.13				347.3	1.009(30)	liq.	misc.	misc.	an alkaloid from tobacco, lethal dose 3-5 mg. per kg. dog, paralyzing nervous system.	
Nile blue sulfate	336.21									is a vital stain.	
Nitrate of sodium	178.09	334-49				colorl.	s.		s.a.	gives a blue color with compounds containing free amino and carbonyl groups.	
Nitric (aero)	85.01	308			4.380	2.267	trip. col.	73(0), 180(100)	s.a.	large doses may cause methemoglobin formation, used to brighten color of meat.	
Nitro-amino guaiacol	184.98									lethal dose for white mice 0.73 mg. per kg., said to be increased by thyroid.	
Nitroguanine	227.08	15			360 expl.	1.801	oil-yel. liq.	0.12	25	misc.	an indicator for determining pH of saliva and urine with Dubocq colorimeter.
n-Nitrophenol	138.08	66			194.0(70)	1.485	tab.	s.h.	v.s.	v.s.	causes vasodilation and fall in blood pressure, dose 0.5 mg. capsule.
o-Nitrophenol	138.06	45.0			214.5	1.447	prisms	v.s.a.s.c.	v.s.	v.s.	an indicator.
p-Nitrophenol	138.08	114			279.2		monocl.	s.h.	v.s.	v.s.	an indicator for pH of blood and sea water with Dubocq colorimeter.
Norleucine	131.11	380									an amino a. said to occur in casein but the obtainable product is cysteine.
Novosamine	585.7	194				colorl.	s.	s.			procaïne, a synthetic substitute for cocaine, rapidly destroyed in body.
Nuclein											a compound of protein and nucleic a. due to hydrolysis of nucleoprotein.
Nucleoproteins											are toxic and antigenic.
Bacterial											occurs in barley embryos.
Barley					lit. cryst.						occurs in non-nucleated as well as nucleated erythrocytes.
Erythrocyte							s.	s.	s.		occurs in gastric juice and mucosa.
Gastric								i.			yields aluphen, guanine, adenine, cytosine, and thymine.
Herring sperm											yields adenine and hypoxanthine.
Leucocyte							s.	i.	i.		yields xanthine, hypoxanthine, adenine, and guanine.
Liver											yields adenine, guanine, thymine, and cytosine.
Mammary											is usually contaminated with tryptin.
o-Pancreas											is richer in P than o pancreas nucleoprotein.
p-Pancreas											yields salicin, guanine, hypoxanthine, xanthine.
Salmon sperm							s.	i.	i.		yields adenine, guanine, thymine, and cytosine.
Spleen											yields uridine and a nucleic a. similar to that from thymus.
Sturgeon sperm											contaminates thyroglobulin.
Thymus											yields adenine, guanine, cytosine, and uracil.
Thyroid							i.				yields adenine, guanine, cytosine, and uracil.
Wheat											occurs as ester in oocypical gland, spermatid, whale, and linseed oil.
Yeast											occurs as esters in essential oils of <i>Heracleum</i> and <i>Passiflora</i> .
Octadecyl alcohol	270.3	59			210.5(15)	0.8124(15)	had.	i.	s.	s.	an albuminoid lining tubes of worm <i>Onchocerca</i> .
o-Octyl alcohol	130.14	-15.3			194.0	0.827	liq.	s.	s.		
Onchocerca	729							s.			

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Orcinol	570.20				red-br. powd.		s.	s. alk.	is a dye.
Orcinol	124.06	108	290	1.39	prisms				an aromatic antiseptic from lichens.
Ornithine	182					s.	s.		an amino acid produced by the action of arginase on arginine.
Oryzanic									a glutelin from rice.
Osealalbuminoid						s.			is the albuminoid other than collagen of bones.
Oshain	588.35	185			quad.	s.h.		s.	crystalline strophanthin with action like digitalis.
Ovoglobulin						s.			a protein of egg white precipitating on dialysis
Ovokeratin									the membranous lining of shells of birds' and sharks' eggs.
Orycea	43	-219.4	-192.0	1.439(0)	gas, liq., hex.	4.89 cm. <sup>3</sup> (0)	2.78 cm. <sup>3</sup> (35)		forms 24% of the volume of dry air.
Oxyhemoglobin	16,869								contains 1.24 cc. O <sub>2</sub> per g. blood of men contains about 17% and women 15.5%.
Palladium	106.70	1555	>2200	11.49(22.5)	rub.	i.	i.	i.	is used to coat hydrogen electrodes, 80 mg. Pd(OH) <sub>2</sub> injected once a week into fat tissue destroys it.
Papaverine	338.17	147	d.	1.337	col. need. f.s.	s.h.	v.s.; a. chl.	0.39(10)	an opium alkaloid, lowers tone of gut and uterus and blood pressure; dose 0.05-0.06 g.
Parformaldehyde	(30.00) <sub>n</sub>	100			wh. amor. powd.	v.s.	i.	i.	lethal dose 99 g., methylates some amino acids in the body.
Parhistone						s.			from thymus contains 4 times as much S as thymus histone.
Paribistidine	182.09	10.5	124	0.894	colorl.	10			3-15 cc. per br. acts as a narcotic, lethal dose 180 cc.
Parosomulin	306.19	193-9			r. leaf.	i.	s.	s.	basic fuchsin, stain for nuclei, elastic tissue, certain granules, nuclei of nerve cells.
Pectocellulose									raw flax, yields pectic acid and cellulose.
p-Palmaraldehyde	148.19		85-89(13)	0.838(16)					is found in citron.
Pelargonidin	304.09								occurs as glucoside in <i>Pelargonium</i> and other flowers.
Pelargonin	630.8	136d.			need.	s.	s.l.s.		pelargonidin glucoside, the antheoyan color of <i>Pelargonium</i> , dahlia, and other flowers.
Pelletierine	141.13		195d.	0.868	oil. liq.	5	misc.	misc.	a monoglycerate alkaloid 15-30 mg. (or better 0.3-1 g. tannate) internally for tapeworm.
Pentacene	332.4	54	324(40)	0.779(20)					a paraffin from waxes.
Pentagallicyl glucose									Chinese nut gall tannin.
Pepsin									is formed by action of HCl on pepsinogen of gastric glands, is supposed to be albumin
Perrin	307	159							a pyrimidine from sperm of yellow perch and wall-eyed pike containing 78% arginine.
Peristatin	214					s.	s.	i.	a purgative glucoside from <i>Cassia sagrada</i> .
Potassiumate of K.	158.03	240d.		2.768(9.9)	rhom.b.	32.38(75)h.			1/1000 solution is used as antidote for morphine and HCN and as uretiral antiseptic.
Peruon	338.05								a barbiturate hypnotic, dose 7 mg. per kg. intravenous.
Persulfox	210.11	110					s.l.s.		a ketolactone produced by aerobic bacteria from persulfox from <i>Leucon persea</i> leaves.
Phasein									an albumin from beans.
Phaseolin									a globulin from kidney and navy beans, deficient in cystine.
Phenacetin	180.08	171	360		y. need.	v.s.l.s.	2c.	s.l.s.	the base of a series of dyes including neutral red.
Phenacetic acid	232.11	174			wh.	s.l.s.	s.	s.	luminal, a hypnotic used especially in epilepsy.
Phenol	94.09	41.0	182	1.070(20)	col. need.	6.71(6)w (8)	misc.	v.s.	produced by putrefaction in the gut, normal blood contains 1-3 mg. per 100 cc., lethal dose 8.5 g.
Phenolphthalein	318.11	361		1.377(32)	trich.	s.l.s.	s.	s.l.s.	a cathartic and indicator.
Phenol red	384.27				r. powd.	s.l.s.	v.s.l.s.	v.s.l.s.	for kidney function test and indicator.
Phenanthrene	194.14	180	371d.		leaf.		s.l.s.		the base of a series of dyes including methylene blue.
p-Phenylenediamine	108.08	130.7	367		colorl. s. f.h.	s.	s.	s.	p-diaminobenzene, a widely used dye and indicator; lethal dose 20 mg. per kg. rabbit.
Phenylethanolamine	137.10								related to adrenaline and with similar action, lethal dose 1 g. per kg. guinea-pig.
Phenylhydrazine	108.08	11.9	245.5 s.d.	1.007(20)	yel.	s.l.s.	misc.	misc.	used to destroy erythrocytes and for forming osazones in sugar analysis.
Phenyl urethane	156.10	51.5-2			need.	v.s.l.s.	v.s.	v.s.	is an anesthetic.
Phloridzin · 2H <sub>2</sub> O	472.22	104-9 (170d. sch.)		1.430	need.	0.1; v.s.h.	v.s.	v.s.l.s.	a glucoside of apple root bark; 5 mg. intravenous produces glycosuria.
Phloroglucinol	138.05	219	subl. d.		rhom.b.	v.s.	v.s.	v.s.	the aglycone of phloridzin, used in test for pentoses.
Phlogene	68.91	<-75	8.2		gas	dec.	dec.		a war gas which forms HCl in the lungs; 0.5 mg. will kill a dog; 1/25,000 is irritating.
Phosphates									4.5 mg. acid soluble per 100 cc. blood (as P <sub>2</sub> ), 1-3 mg. P per 100 cc. spinal fluid, deficiency causes rickets.
Phosphoric acid	316								occurs in muscle, and is hydrolysed during contraction.
Phosphorus	124.08	44.1 (99.34)	380	1.82	hex.	s.l.s.h.	0.3	s.	lethal dose of yellow P 30-100 mg. causes cirrhosis of liver, antidote 100-200 mg. CaSO <sub>4</sub> every 2 min.
Phrenosin	227.75				wh. powd.				a phantoid of the brain.
p-Phthalic anhydride	148.03	130.3	261.5	1.327(4)	col. pr.	v.s.l.s.	s.	s.l.s.	the base of a series of dyes used as indicators.
Phycocyanin					blue				a blue protein of blue-green algae active in photosynthesis.
Phycocyanidin									a red protein of brown and red algae active in photosynthesis.
Phylloerythrin									a tetraglycosyl mg-free photosensitizer from chlorophyll; animals eating buckwheat may be poisoned.
Phylloerythrin					r. prisms		s.l.s.		one of the 4 pyroles obtained by decomposition of hemoglobin and chlorophyll.
Phylloerythrin	116.11	69	89-7		pl.				Ca salt of phytic a. in cereals and other plants.
Phytin	888.42	178	1.102		amor.	s.	s.	i.	a polymer of isoprene and constituent of chlorophyll.
Phytol	266.3								polyestered glucoside occurring in various plants.
Phytosterol	386.49	360			wh. powd.		v.s.	v.s.	an alkaloid from <i>Pilocarpus</i> increases saliva and sweat, constricts pupils, decreases intraocular tension.
Pilocarpine	298.14	94			col. need.	v.s.	v.s.	s.l.s.	a bicyclic terpene from turpentine, it is toxic and excreted as glycuronate.
Pine (C <sub>10</sub> ) (a)	136.13	-55	184	0.878	col. liq.	v.s.l.s.	misc. abs.		diisopropylterpene; dipeptide anhydride, amino a. anhydride, occur in fibroin (silk).
Piperazine	114.09								

	M.W.	M.P.	E.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Piperidine.....	85.09	-9	165.8	0.869(20)	oil. liq.	misc.	misc.	.....	a vasodilator and the base of a series of alkaloids.
Piperine.....	253.16	124.5	.....	.....	oil. monool.	v.a.s.	6.7; 50(90)	2.8	an irritating piperidine derivative from pepper, is used in laxative pills.
Piscagery alcohol.....	200.22	73	.....	.....	stale	.....	.....	.....	occurs as ester in pisang wa (wild banana).
Platinum.....	195.23	1755	4950	21.45(20)	oil. silv. met.	i.	.....	.....	used for passing through glass in making electrodes, the solid sol. and salts toxic.
Potassium.....	39.10	63.3	769	0.869(20)	oil. silv. met.	dec. to KOH+H <sub>2</sub>	s.	.....	20 mg. per 100 cc. serum 400 mg. per 100 cc. corpuscles, stimulating ion antagonized by Ca.
Populin-2H <sub>2</sub> O.....	430.20	180 sol.	.....	.....	oil. need.	0.66c.	s.	s.	a salicin benzoate glucoside in poplar.
Primeverose.....	312.15	200-10	.....	.....	cryst.	s.	s.	i.	a gluco-xyloside combined with an aglycone in cornels and primroses.
Prothavine.....	307.1	.....	.....	.....	need.	s.	s.	i.	the acid sulfate homologous of acetylthavine, an acetylthavine dye used intrav. in sleeping sickness.
Protein from Oats.....	.....	.....	.....	.....	.....	i.	i. abs.	.....	has a higher cystine and histidine content than gliadin and borden.
Sorghum.....	.....	.....	.....	.....	.....	i.	s.	.....	contains no tryptophan.
Proline.....	115.08	205.00	.....	.....	.....	s.	s.	i.	an amino a. from proteins, does not react with nitrous acid or anhydride, is a sugar-former.
Propand.....	60.06	-137	97.4	0.894(20)	liq.	misc.	misc.	misc.	is 3 times as intoxicating per mol as ethanol.
Protasasin.....	255.14	123	.....	.....	need.	s.	s.	i.	racemic mandelonitrile glucoside from cherry laurel.
Prunasin.....	255.14	147	d.	.....	need.	.....	s.	.....	l-mandelonitrile glucoside from Prunus.
Pyridosteryl alcohol.....	493.54	69-68	.....	.....	.....	i.	i.	i.	occurs as ester in wax of plant leaves <i>Populus</i> .
Pyridon.....	132.10	.....	201.2	0.892	liq.	i.	misc.	misc.	a terpene causing fatty infiltration of liver, heart, and kidney.
Pyrene.....	120.08	217	d.	.....	misc. need. f.i.d.	v.a.	s.	v.a.s.	the type compound of the di-aromatic bases in nucleic a.
Pyropin.....	260.06	255	d.	.....	red need. f.i.d.	s.	s.	s.	a dye which is said to stain Ca precipitates in cell contents.
Putrescine.....	88.108	27	183	.....	colorl.	.....	.....	.....	a pyrazine arising from putrefaction of ornithine and occurring in some mushrooms.
Pyridine.....	79.05	-43	115.3	0.893	liq.	misc.	misc.	misc.	the base of a series of alkaloids, occurs in heated biological products, used in medicine although toxic.
Pyrimidine.....	80.05	22	124	.....	cryst.	s.	s.	.....	the base of uracil, thymine and cytosine in nucleic a.
Pyrogallol.....	120.05	133	260d.	.....	need. or fl.	v.a.	100(26)	v.a.	is used as a reagent in analyses and photography and to absorb oxygen.
Pyrophenol.....	71.08	.....	87.3-8.5	0.883(22.5)	liq.	misc.	misc.	misc.	the base of proline and some alkaloids.
1,4-Pyrene.....	90.08	32.5	217.7	1.19(40.3)	pr.	v.a.s.	s.	v.a.	is the base of melanin a. and other compounds and related to chromone.
Quercetin.....	302.08	310d.	subl.	.....	yel. need.	0.33	0.48	.....	a yellow dye occurring as a rhamnoside in oak bark.
Quercitol.....	164.09	246d.	.....	1.585(10)	monool.	v.a.h.	i.	i.	a cyclohex found in oak bark.
Quercitrin.....	495.17	135	.....	.....	yel. need. or fl.	v.a.s.	s.l.a.	0.8	a rhamnoside of quercetin in oak bark, tea leaves, and other plants.
Quinidine red.....	441.12	.....	.....	.....	.....	.....	.....	.....	an indicator for determining pH of stomach with Duboseq colorimeter, pK <sub>a</sub> = 2.73 at 20°.
Quinoline.....	128.06	-19.5	337.7	1.088	oil. liq.	s.l.a.	misc.	misc.	obtained from bone oil and petroleum.
Quinhydrone.....	218.08	171.0	subl.	.....	dk. pr. fr.	s.h.	v.a.	v.a.	used in determining pH; the difference between the quinhydrone and H <sub>2</sub> electrodes is 0.704 v.
Quinine.....	324.20	175.01	.....	.....	alky need. f.h.s.	0.687(26)	165	22	alkaloid from cinchona bark specific for malaria, lethal dose 5 g.
Quinone.....	108.08	114.7	subl.	1.31	yel. pr. f.w.	s.l.a.	v.a.	v.a.	a powerful antiseptic, secreted by skin of land turtles, excreted as hydroquinone glycoside.
Radium.....	226.97	980	1140	5.0(7)	silv.-wh. met.	d. ev. H <sub>2</sub>	.....	.....	extremely toxic and deposited in bones, some excreted by bowel, said to have action like K.
Raffinose.....	334.25	119	130d.	1.465	need.	14.0(20)	v.a.	.....	fructose, glucose, galactose-trisaccharide from <i>Boeastricta</i> , sugar beet, cottonseed, and other plants.
Racemid blue.....	495.83	.....	.....	.....	.....	.....	.....	.....	a microchemical stain.
Racemidol.....	110.08	110	275.5	1.285(15)	oil. tab.	v.a.	v.a.	v.a.	found in ascorbic acid and vitamin preparations, an antiseptic used in medicine, lethal dose 12 g.
Respiratory enzyme.....	.....	.....	.....	.....	.....	i.	i.	i.	a heme compound in cells, its union with CO is broken by illumination.
Reticinol.....	.....	.....	.....	.....	.....	i.	i.	i.	fibers of reticular tissue not digested by pepsin or trypsin, does not yield gelatin on boiling.
Rhamnose.....	164.09	135-40	.....	.....	colorl.	v.a.	v.a.	v.a.s.	a trisaccharide: galactose-rhamnose-rhamnose from Persian berry, <i>Rhamnus</i> .
Rhamnetol.....	196.11	121	.....	.....	trid. pr.	v.a.	v.a.	v.a.s.	a pentoside sweet alcohol with the addition of a methyl group on the end of the C chain.
Rhamnose.....	164.09	126	1.471	.....	colorl. f.w.	57(18)	s.l.a.	i.	a methyl pentose from rhamnose and various glucosides.
Rhodose.....	164.09	144	.....	.....	.....	.....	.....	.....	a methyl pentose said to be the optical antipode of fucose.
Rhodinol.....	158.11	.....	112-14	0.861(20)	.....	v.a.s.	misc.	misc.	an open chain terpene from citronella, geraniums, and rose (synthetic oil of rose).
8-Rhodose.....	150.08	87	.....	.....	.....	.....	.....	.....	the pentose sugar of nucleic a.
Ricin.....	150.08	.....	.....	.....	.....	s.	i.	.....	the toxic protein of castor bean; 180 mg. by mouth is fatal to man, agglutinates r.b.c.
Ribonose.....	164.09	.....	.....	.....	.....	.....	.....	.....	galactose-rhamnose-rhamnose trisaccharide combined with ascorbin in <i>Ribonin</i> .
Rosacetal.....	210.10	.....	d.	.....	need.	s.l.a.	s.	i.	a dye.
p-Rosacetal.....	315.18	188-9	.....	.....	lax.	i.	s.	s.	basic fuchsin, a stain for mucin, elastic tissue, granules, and nuclei of nerve cells.
Rubidium.....	85.44	38.5	700	1.392(19.4)	silv.-wh. met.	dec.	s.	.....	a stimulating ion found in organisms, stimulates respiratory center, acts like K.
Rutin.....	610.24	190	.....	.....	need.	s.l.a.	v.a.	.....	a hydroxyflavone gluco-rhamnoside in cornell, potato, tomato, tobacco, California poppy.
Saccharin.....	183.11	230d.	.....	.....	oil. monool.	0.48(25)	3.1	.....	500 times sweeter than sucrose.
Salicin.....	290.14	201.5	240	1.494(26)	oil. leaf.	3.6(15)	s.	.....	saligenin glucoside from willow, poplar, <i>Spirea</i> .
Salicyl alcohol.....	124.06	30.8	.....	1.11(20)	rhomb.	s.	v.a.	v.a.	saligenin, 0.5-1.5 g., 4-6 t.p.d. in rheumatism and gout, local anesthetic d.b.
Salicyl aldehyde.....	122.06	-10	199.5	1.178(24)	.....	s.l.a.	v.a.	v.s.	an antiseptic from <i>Spirea</i> , used as a chemical reagent.
Salicin.....	290.14	.....	.....	.....	.....	.....	.....	.....	protein from salmon sperm, 8.4% arginine, not digested by pepsin.
Santalin.....	246.14	170	1.187(20)	oil. pr.	0.62c.; 0.4h.	2.3	1.5	.....	an antihelmintic from flower buds of <i>Artemisia</i> , dose 0.2 g. orally.
Saponin.....	726.5	.....	.....	.....	.....	s.	i.	i.	one or more glucosides reducing surface tension, erythritol, emetic.
Sarcosine.....	88.06	210d.	.....	.....	rhomb.	v.a.	s.l.a.	i.	methyl glycine from decomposition of creatinine.
Sarsapogenin.....	333.376	248	.....	.....	.....	s.	s.	i.	sarsapogenin glucoside from <i>Sarsaparilla</i> , emetic, expectorant and emetic.
Sarcolin.....	370.30	151-48	.....	.....	r. powd.	i.	s.l.a.	s.l.a.	a fat dye, promotes granulation in wounds.
Sarcolin red sulfonate.....	402.38	.....	.....	.....	.....	s.	s.l.a.	s.l.a.	a fat dye, promotes granulation in wounds, stains cells of Langerhans.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Scombrin									a protein from mackerel sperm, 88.6% arginine.
Scombrone						a.			a histon from immature mackerel sperm.
Scolecimane	303.17	33-3			cryst.			a.	a hypotonic alkaloid from <i>Scolecia</i> root.
Sec-isoamylalcohol	172.19	13	228-29	0.827 (35)					is found in oil of rose.
Secotine									a substance set free by food entering the duodenum, causing pancreas to secrete.
Sedobaptene	210	180-5							a leucotoxin from <i>Sedum spectabile</i> .
Semioarbutide	75.06	96							a base which reacts with aldehydes and ketones forming semicarbazones.
Serine	105.06	228d			hex. tab.	a.	i.	i.	$\beta$ -hydroxy alanine first found in silk gum, occurring in most proteins.
Serum albumin	65,000								is considered the same as the albumin of nephritic urine.
Serum globulin									is difficult to free from albumin but the ratio of the two has been much studied.
Silicon	35.06	1430	2600	2.40 (30)	cub. gray	i.			the vitreous humor of eye contains 0.06% (30); dry basis; feathers contain it organically combined.
Silver	107.88	960.5	1850	10.5	cub. wh. met.	i.			both metal and ions are antiseptic; the low requires AgNO <sub>3</sub> in babies' eyes at birth.
Sinigrin	397.36	126			need.	v.a.	a.s.		allyl isothiocyanate glucoside from seeds of black mustard.
Stosterol	386.55	140			hexag.	i.	a.	a.	a steroid widely distributed in plants not absorbed from alimentary canal.
Stasole	131.08	85	266.30		H.I. lgr.	0.05	v.a.		a bad-smelling ptomaine from putrefaction of tryptophan, reduces h.p., lethal dose 5 mg. sub. cu.
Sodium	23.00	97.5	880	0.871 (30)	cub. met.	dec. to NaO	a.	a.	a stimulating ion, 535 mg. per 100 cc. serum, 43 mg. per 100 cc. corp., 1% NaCl physical. salt sol.
Somnifone									equal parts of barbitol and diethylamine diallylthiothiuronate, dose 5 cc. in vena.
<i>s</i> -Sorbidol	191.12	110 amh.			color. need.	a.	v.a.s.		hemolytic sweet alcohol found in mountain ash berries and other plants.
<i>s</i> -Sorbose	180.09	154			rhomb.	a.s.	a.s.	a.s.	is produced by action of <i>B. nystaceus</i> on sorbitol.
Spartine	204.23		335.3d.	1.028 (30)	oil. oil	v.a.s.	v.a.	v.a.	an alkaloid from <i>Spartium</i> causing muscular spasms.
Spermine	302.54								bis-aminopropylaminebutane.
Sphingomyelin	1035.00	104-4			wh. need.				a phospholipid from the brain.
Sphingosine	265.28	244	258d.		cryst.	i.	a.	a.	a base from sphingomyelin and phosphenin, excreted unchanged, purple with Cu <sup>++</sup> and sugar.
Spongein						i.			the protein of bath sponge yielding diiodotyrosine and bromine.
Stachydrine	140	210			cryst.	a.	a.	i.	the betaine form of protine from <i>Stachys</i> and other plants.
Stachyrose	759.535	170			rhomb. pl.				fructose-glucose-glucose-glucose tetraaceticamide murexide from <i>Stachys</i> and other plants.
Starch	(162.58) <sub>n</sub>	d.		1.5 (21)	wh. amorph.	i.	i.	i.	the chief polysaccharide yielding glucose in plants, center of grain glucose phosphate.
Stercorin									is the normal pigment of feces, said to be urobilin.
Stigmastanol	440	140							a sterol from oyster beans and vegetable oils.
Stilobolin									the globulin of Chinese velvet beans precipitating between 40-60% acet. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .
Strontium	87.63	750 (900)	1150	2.40	cub. y. met.	dec.	a.		occurs in bones, teeth, soft parts, action similar to Ca but causes rickets when substituted.
Styrylamine	504.19	266d.	270 (5)		terr. f.al.	0.016 (25)	0.9	0.013	an alkaloid from <i>Stylococcus</i> ; 5 mg. increases perception of light 100%, i.d. 0.75-4 g., changes chloro- acid.
Sturin									a protein in sturgeon sperm, which approaches histon in nature.
Styrene	94.217	185	1.588 (15)		color. monoc.	179 (0), 497 (100)	0.9		a waterproof constituent of cork considered estrobolactone in nature.
Styran III	361.80	185			r. br.	i.	a.	a.	the most common disaccharide of plants, rapidly hydrolyzed in the gut.
Sulfate of Mg.	120.38	1185		2.66	col. cr.	36.0 (0)		1.18 (15)	a fat dye which when fed with fat is deposited with it, shows up laterals.
Sulfonal	228.34	128	300d.		prisms	2 (15), 6.7 (100)	50 habs.	a.s.	an osmotic cathartic increasing bulk of material in rectum causing defecation.
Sulfur	32.06	ca. 20	444.6	1.930	pa. yel. amorph.	i.	i.	i.	0.5-1 g. is a hypotonic.
Sulfamphenamine	267.7				o. yel.	v.a.			occurs as sulfate, thiocyanate, ethereal sulfur and protein sulfur in body.
Synthalin	256								less irritating on intramuscular or hypodermic injection but less efficient than other arphenamines.
Synthalin B	266								diamethylglycine formerly used as a substitute for insulin.
Synthesin	256								dioctamethylglycine formerly used as a substitute for insulin.
<i>s</i> -Tagatose	180.09	124				i.	i.	i.	acid nonprotein from muscle tissue.
<i>s</i> -Talinol	133.11	99				v.a.			a non fermentable ketohexose.
<i>s</i> -Talos	180.09								a hemolytic sweet alcohol.
Tantalum	181.40	2,650	ca. 4100	16.6 (met.) 14.461 (powd.)	cub. blk.-gr. met. or bl. powd.	i.	i.	i.	a hexose.
Terchamyl alcohol	718.83	81			silvery	i.	a.s.	i.	used for surgical instruments may be sterilized in flame without losing hardness.
Taurine	125.07	88	d.		terr. need.	6.8 (12)	i.	i.	occurs in leaves of <i>Turkocordine</i> .
Terpia lactate	199.17	anh. 117.1			col. rhomb.	0.3 (25)	10	v.a.	occurs in bile in combination with cholic acid.
Terpineol	154.14		193	0.855 (30)	col. liq.	i.	mic.	mic.	an open-chain terpene used as cough medicine, dose 0.1-1 g., excreted as glycuronate.
$\alpha$ -Terpinol	154.14	35-40	219.8	0.834	col.	i.	v.a.	v.a.	a terpene from essential oils, changes hemoglobin to methemoglobin.
$\beta$ -Terpinol	154.14	33	210	0.820 (35)		i.	v.a.	v.a.	a terpene with like odor, causes fatty infiltration of liver.
$\gamma$ -Terpinol	154.14	70	218	0.830 (30)		i.	v.a.	v.a.	a terpene with like odor, causes fatty infiltration of liver.
Tetraethoxyethylene	165.83	-22.4	130.8	1.633	col. liq.		mic.	mic.	a terpene with like odor, causes fatty infiltration of liver.
Tetraethoxyphenyl-phthalin, tetraethoxy-tetra-									
iodo									
Tetraethyl lead	323.36		302	1.83					used as liver function test dose 5 mg. per kg.; 4 hr. prior for gall-bladder, X-ray dose 0.1 g. per kg.
Tetra-methyl panto-									in poisoning accumulates in brain, slowly liberates lead and induces lead poisoning.
thion	481.75	180				2.58 (20)	20 (20)	i.	occurs in methyl violet, used as indicator, antiseptic for Gram & B., microscopic stain.
Tetronal	559.38	65				a.	a.	a.	a hypotonic, may cause hematuria and pyuria to appear in the urine.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Thallium	204.39	303.5	1860	11.85	tetr. bl. wh. met	i.	i.	i.	very toxic, used as a rodent poison, locally to remove hair but may become general by absorption.
Thaloxine	311.17	193	.....	1.305	gilt. pr. f.al.	v.a.s.	10; v.s.a.	0.7(10); v.a. chl.	an opium alkaloid and convulsive poison, lethal dose 0.45 g.
Thalobromine	300.09	327	subl.	.....	rhomb. f.w.	0.03(15), 0.67(100)	0.030(17)	0.95	dimethyl caffeine from coons, similar physiologically but less soluble in water.
Thaophylline	180.09	327	.....	.....	need. f.w.	0.44(15), 1.3(37)	s.l.s.	s.l.s.	an isomere of theobromine and greater diuretic than caffeine; dose 0.5 g.
Thioazotate of Na	81.07	362.3	.....	.....	col. rhomb. deliq.	s.; d.h.	.....	.....	occurs in the body, more easily detected in saliva.
Thionin	227.15	.....	.....	.....	gr. powd.	s.	s.	.....	a vital and metachromatic stain.
Thiosulfate of Na <sub>2</sub> H <sub>2</sub> O	248.16	d.6	.....	1.685	monocl. col.	74.7(3), 301.5(300)	.....	.....	used intravenously as an antidote to heavy-metal poisoning.
6-Thiouracil	120.06	.....	.....	.....	.....	.....	.....	.....	is known only from its derivatives.
Thujone	153.13	.....	300	0.9130(30)	.....	.....	.....	.....	a terpene occurring in Thui, wormwood, and sage oils.
Thymene	136.13	.....	195	0.847(14)	liq.	.....	.....	.....	.....
Thymine	126.05	.....	.....	.....	.....	.....	.....	.....	.....
Thymol	150.11	51.5	221.8	0.899	col. plates	0.030(15)	v.s.	v.s.	a pyrimidine base from uric acid.
Thymol blue	445.27	.....	.....	.....	.....	.....	.....	.....	thymol camphor, an aromatic veratriglycoside; dose 3 g. per day, lethal dose 9 g.
Thymus histon	6000.00	.....	.....	.....	.....	.....	.....	.....	an indicator pKa 1.5 and 8.8.
Thymin	.....	.....	.....	.....	.....	.....	.....	.....	injected in vein lowers blood pressure and leucocyte count and coagulability.
Thymin	.....	.....	.....	.....	.....	.....	.....	.....	protein from tuna fish, 78.9%; arginine N.
Thyroglobulin	.....	.....	.....	.....	.....	.....	.....	.....	a pseudoglobulin of the colloid containing thyroxine and diiodotyrosine, is thyroid hormone.
Thyroxine	776.92	321-3d	.....	.....	need.	.....	i.	i.	1 mg. raises basal metabolic rate about 5% in 40 hrs., is 65% iodine.
Tin	118.70	321.9 sub.	2270	5.75	cubic gray	i.	i.	i.	is toxic but poisoning rare due to insolubility, eliminated by gut.
Tissue fibrinogen	.....	.....	.....	.....	.....	.....	.....	.....	45% albumin, 55% globulin, extracted from lung, 3 cc. 1% solution orally halves clotting time.
Thiamin	47.90	1800	>3000	4.9(10)	cubic gray	i.; d.h.	i.	i.	occurs in body, 5% of sulfate is antiseptic.
Toluen	92.06	-95.1	110.5	0.860(20)	col. liq.	i.	misc.	misc.	occurs in coal tar, is antiseptic.
Tonalbumin from coles vesum	.....	.....	.....	.....	.....	.....	.....	.....	lethal dose 4 mg. per 50 kg., hemolytic, 1 cc. Calmette antivenom neutralizes 1 mg. tonalb.
Trans terpin	154.14	.....	265	.....	.....	.....	.....	.....	like oil, produces fatty infiltration of liver.
Trihalos	245.17	210	.....	.....	.....	.....	.....	.....	glucose disaccharide; occurs in mushrooms and other fungi; used in differentiating paratyphoid.
Triacetin	218.11	-75	350	1.161	col. liq.	7.17	misc.	misc.	occurs in butter, cod-liver oil, and Elaeagnus seeds.
Trifluoromethanol	282.77	80	.....	.....	.....	2(37)	.....	.....	avertin, 80-100 mg. per kg. for colonic anesthesia.
Trihydroxy	202.20	<-75	310	1.267(20)	.....	i.	v.s.	v.s.	has a very repulsive taste.
Triacetin	254.48	31.1	.....	0.821(40)	trid.	i.	s.l.s.	s.	.....
Triacetin	286.26	-85	.....	0.868(15)	color.	s.l.s.	s.	s.	fed to rats is deposited in fat tissue.
Triacetin	470.26	8	.....	0.864	.....	.....	.....	.....	.....
Triacetin triphosphate	338.21	.....	.....	.....	.....	.....	.....	.....	is the chief agent of "ginger paralysis"
Triethylarsanil	400.19	.....	.....	.....	.....	.....	.....	.....	Hoffman's violet is used in staining amyloid.
Triphenylamine	337.06	216d	.....	.....	prisms	v.s.	s.l.s.	i.	occurs in many seeds, appears in urine after taking nictotina.
Triphenylamine	458.57	44.5	.....	0.891(15)	.....	.....	.....	.....	obtained by vacuum distillation of laural oil.
Triphenylamine oxide	59.06	-124	2.5	0.822(-5)	color.	v.s.	v.s.	s.	is formed by putrefaction of choline and other bases, 0.001-0.002% in blood, used in medicine.
Triphenylamine oxide	75.0	96	.....	.....	.....	.....	.....	.....	occurs in muscle and urine.
Triphenylamine	107	.....	.....	.....	prisms	.....	.....	.....	occurs in mushrooms.
Triphenylamine	229.21	250	.....	.....	prisms	.....	.....	.....	occurs in blood, 7.5 mg. per 100 cc., in pig's blood 10 mg. per 100 cc., in corpses only.
Triphenylamine	300.11	355d	.....	.....	.....	.....	.....	.....	in seeds of Erythraea, 12-15 mg. produces prolonged tetanus in frog.
Triphenylamine	327.08	81.7	340 enl.	1.054(20)	col. monocl. f.al.	0.10(15)	v.s.h.	s.l.s.	explosive, lethal dose 0.5 g. per kg., extracted as diminoacrylate glycerate.
Triphenylamine	334.8	-17	340(15)	0.915	.....	i.	s.l.s.	v.s.	the chief ingredient of olive and many other oils.
Triphenylamine	394.23	79	.....	.....	col. tab.	0.3	v.s.	v.s.	a hypnotic, action prolonged, produces porphyria.
Triphenylamine	494.76	45, 65.1	310-520	0.860(80)	need.	i.	0.004(21)	v.s.	occurs in ordinary fats, especially palm oil.
Triphenylamine	490.80	54.5, 70.8	.....	0.860(80)	flakes	i.	v.s.	s.	occurs in tallow and other fats.
Triphenylamine	141.13	63	353	1.069	need.	v.s.	v.s.	v.s.	an alkaloid, diminishes systolic amplitude of heart, lethal dose 80 mg. per kg. dog.
Trypan blue	444.54	.....	.....	.....	.....	.....	.....	.....	a trypanoidal dye.
Trypan red	383.47	.....	.....	.....	.....	.....	.....	.....	a trypanoidal dye.
Trypanamide	367	.....	.....	.....	wh.	v.s.	.....	.....	used in trypanomiasis and early tertiary syphilis, lethal dose 6 g.
Trypanol	304.11	289d	.....	.....	col. leaf.	s.l.s.; v.s.h.	s.l.s.	i.	an essential amino acid destroyed by acid hydrolysis of protein.
Trypanol	101.1	50	.....	.....	monocl.	s.	v.s.s.	v.s.s.	produced by putrefaction of proteins, gives yellow-brown color with Millon's reagent.
Tuberculin	.....	.....	.....	.....	.....	.....	.....	.....	is said to be a protein.
Tuberculin	.....	.....	.....	.....	.....	.....	.....	.....	potato globulin, nutritive value 71% of milk proteins.
Tungsten	184	2400	3530	18.7	blk.	i.	i.	i.	is used as phosphotungstic acid to precipitate proteins and alkaloids.
Turpene	242.18	.....	.....	.....	.....	.....	.....	.....	fructose-glucose disaccharide may be obtained from melastoma.
Tyrosine	173.5	161	.....	.....	her.	v.s.	.....	.....	from ergot or salivary poison of ophiophaga, from putrefaction of tyrosine, contracts uterus and arteries.
Tyrosine	151.09	205d	.....	1.459 cm. silk	need.	0.50(100)	0.01(17), i. abs.	i.	essential amino acid, absorption band at 2800 Å, oxidation produces colored products.
Tyrosol	138.1	80	310	.....	rhomb.	.....	.....	.....	from putrefaction or yeast fermentation of tyrosine.
Umbelliferone	192.04	327	subl.	.....	need.	1.0(100)	s.	s.l.s.	obtained by dry distillation of resins causes fluorescence of eye humors and acid-base titrations.
Uracil	112.05	238	.....	.....	cr. powd.	v.a.s.; v.s.h.	i.	s.	a pyrimidine base in uric acid.
Uracium	238.14	<1850	ign.	18.7 cub.	wh. met.	i.	i.	i.	produces nephritis, lethal dose 3 mg. per kg. dog or rabbit.
Urea	60.05	112.7	d.	1.335	tetr.	v.s.	sc.	s.l.s.	in uremia more than 100 mg. per 100 cc. blood, 3% in shark's blood.
Urea	.....	.....	.....	.....	.....	s.	s.	i.	said to be a crystallizable protein but a protection colloid is necessary, widely distributed.

	M.W.	M.P.	B.P.	D.	Crystal form Color	Solubility in 100 cc.			Physiological
						Water	Alcohol	Ether	
Urethane.....	80.06	48	100	1.11( $\frac{4}{3}$ )	col. need. f. lq.	v.s.a.	v.s.	v.s.	1 g. per kg. produces anesthesia.
Uridin.....	244.13	150							small d-riboside, a nucleoside from nucleic a.
Urobilin.....	502.56								produced by putrefaction of bilirubin in gut and excreted in kidney, or removed by liver.
Urobilogen.....									produced by putrefaction of bilirubin in gut and excreted in kidney, or removed by liver.
Urochrome.....						a.	a.s.	i.	is excreted by kidney, amount varies with basal metabolic rate 0.57-1.2 g. per day.
Uverythin.....						a.	a.		ordinarily only small amount in urine, increases under various conditions.
Urotropine.....	140.13	360	d.		rhomb. f.al.	93(12)	3.0	i.	hexamethylenetetramine formed by NH <sub>3</sub> on formaldehyde, hydrolyzed in acid urine as anti-septic.
Valer-lactone.....	100.06		220		liq.	a.	a.	a.	occurs in wood vinegar.
Valine.....	117.09	315d.			leaf.	8.5(15)	i.	i.	intravenous injection lowers blood pressure, occurs in proteins.
Vanadurum.....	50.95	1715	3400	3.035(15)	li. gr. met.	i.	i.	i.	occurs in blood of aciditians, used in medicine.
Vanillin.....	152.08	81	235	1.059	col. need. f.w.; sh.	40; sh.	v.s.	v.s.	occurs in vanilla and other plants; lethal dose 30 g. for rabbit.
Veratrine.....	530.39	205			cryst.	i.	a.	a.	causes delayed relaxation (fibillation) of skeletal muscle.
Vicianose.....	312.15	210d.							glucose-aminose disaccharide, from seeds of vetch Vicia.
Villin.....									globulin from pea, horse bean, and lentil.
Vignin.....									globulin of cow peas.
Vitamin D.....	382.33								irradiated ergosterol, vitamin D, tends to prevent both rickets and osteoporosis.
Vital red.....	410.54								a vital stain.
Vitellin.....									a lecithin-free phosphoglobulin from egg yolk; lecithin is combined with it in the yolk.
Water.....	18.01	0	100	1.000( $\frac{4}{3}$ )	col. liq. or col. hex., cryst.		misc.	a.s.	forms 68% of the body; is necessary for temperature regulation, dehydration causes fever.
Xanthine · H <sub>2</sub> O.....	152.06	>300			yel.-wh. powd.	0.30(17)	0.033(17)	v.s. alk.	produced by action of guanosine on guanidine, occurs in bladder-stones, soil, and urine.
Xanthone.....	186.06	174	351		long need. f.al.	a.s.a.	a.h.	a.s., 1 gr.	the base of certain plant pigments.
Xanthophyll.....	519	174.5			or. quad.	i.	a.	a.	a polymeric of isoprene and related to carotin but containing oxygen, in green and yellow plants.
Xanthochloamin.....	770.34				yel. need.	v.s.	v.s.	i.	hydroxy flavone glucoside from fruit of Rhumex.
Xanthosin.....	305.14					a.s.			xanthin-riboside arising from guanosin by deamination.
Xiphatin.....									prolamine from sperm of sword fish, contains 81.6% arginine N.
Xylan.....									an indigestible polysaccharide of xylose in many plants.
Xylitol.....	152.09	104			need.	a.	a.		a pentahydric sweet alcohol.
Xylose.....	150.08	153		1.335	need.	117(30)	v.s.a.s.	v.s.a.	a pentose widely distributed in plants, fermented in gut yielding lactic and acetic acids.
Yeast phosphoglobulin.....									zymo-enzyme.
Zain.....									the prolamine from corn; contains no tryptophan, cystine nor lysine.
Zinc.....	65.38	419.4	907	7.142	hex. double-cri. met.	i.	i.	i.	occurs in organisms; small amounts stimulate fungi, large amounts are antiseptic.



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